RENAL PROSTAGLANDINS IN EXPERIMENTAL RENAL HYPERTENSION AND IN AUTOREGULATION OF RENAL BLOOD FLOW

BY

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I hereby declare that this thesis was composed by myself alone and that it describes work, some of which was performed in collaboration with various other people, as outlined in the acknowledgements.

ABSTRACT

Accompanying the arterial hypertension in the dog following the partial constriction of one of a pair of renal arteries, there are rises in plasma renin activity, plasma volume and renal venous prostaglandins E and F of the contralateral kidney, reaching maxima on days 2, 4 and 5 respectively. By the tenth day all parameters were normal, with the exception of blood pressure. It is suggested that the contralateral kidney responds to the fluid retention by natriuresis and diuresis mediated by an increase in prostaglandin synthesis. It is also concluded that PGE is unlikely to play a role in the antihypertensive action of this kidney. A transient elevation of circulating PGA was observed in some experiments.

In the two-kidney Goldblatt rat a transient decrease in renal plasma flow of the clipped kidney was seen whilst the contralateral kidney showed no change. Renal venous PGE_2 and $PGF_2\alpha$ concentrations changed inversely with renal plasma flow and calculated secretion rate of the clipped kidney fell, although it was not possible to establish the significance of this fall. Such a fall could lead to a reduction in intrarenal vasoconstrictor potentiation produced by prostaglandins in this species. The absence of a rise in secretion rate of PGE_2 in either kidney raises further doubt concerning renal prostaglandins as mediators of the antihypertensive action of the kidney in the rat.

In the pump-perfused dog kidney calculated secretion rate of PGE fell as perfusion pressure fell below the autoregulation range, whilst PGF secretion did not change. Meclofenamate failed to abolish renal blood flow autoregulation, despite a 70% fall in prostaglandin synthesis and it is therefore concluded that autoregulation is not dependent on prostaglandins.

GLOSSARY OF ABBREVIATIONS

ang	angiotensin
ADH	antidiuretic hormone (vasopressin)
BP	blood pressure
CVP	central venous pressure
ECFV	extracellular fluid volume
GFR	glomerular filtration rate
HR	heart rate
Ht	haematocrit
i.m.	intramuscular
i.v.	intravenous
i.p.	intraperitoneal
IFV	interstitial fluid volume
i.v.p.	intravenous pyelogram
OD	optical density
PAH	para amino hippuric acid
PG	prostaglandin - followed by letter denoting type, e.g. PGA, PGB etc.
PRA	plasma renin activity
PV	plasma volume
RAP	renal artery (perfusion) pressure
RIA	radioimmunoassay
RICs	renal interstitial cells
RBF	renal blood flow
SHR	spontaneously hypertensive rats

"One-kidney Goldblatt" preparations will be the term used to describe hypertensive animals in which the kidney contralateral to the kidney with the renal artery clamp has been removed whilst "two-kidney Goldblatt" preparations will describe animals in which the contralateral kidney is left intact.

DRUGS AND CHEMICALS

Cristamycin (benzylpenicillin with streptomycin, Glaxo)

Evans Blue (Gurr)

Hibitane (chlorhexidine, ICI)

Hartmann's solution (Steriflex No. 11, Allen and Hanbury's)

Kabikinase (streptokinase, Kabi)

Nembutal (pentobarbitone, Abbott)

Nobecutane (BDH)

Pentothal (thiopentone, Abbott)

Pethilorfan (pethidine, Roche)

Polybactrin (polymixin with neomycin and bacitracin, BDH)

Stericol (Sterling Industrial)

Thiocyanate (potassium salt, BDH analar)

Thiomersal (BDH)

CONTENTS

INTRODUCTION

The introduction of a normal kidney into the circulation of dogs with perinephritic cellophane hypertension (Kolff,1958) and one kidney Goldblatt hypertensive rats (Tobian, Schonning and Seefeldt, 1964) rapidly reduces blood pressure (BP) to normal despite replacement of the urine loss. The presence of the untouched kidney in the two kidney Goldblatt dog has been shown to lead to a milder form of hypertension than if this kidney is removed (Goldblatt, 1937; Goldblatt, Kalin and Hanzal, 1939) and this has been interpreted as a protective mechanism of the kidney (Fascioli,1938).

An endocrine antihypertensive action has been implicated since even in the absence of excretion by such maneouvres as bilateral ureterocaval anastomosis, an antihypertensive effect is still seen in the dog (Grollman, Muirhead and Vanatta, 1949).

Muirhead's group has drawn attention to the renal medulla. In the renoprival hypertensive dog (bilateral nephrectomised), autoexplanation of whole kidney and medulla but not of the cortex, prevent hypertension from developing (Muirhead, Jones and Stirman, 1960). In the renoprival hypertensive rat, rabbit medullary extract is antihypertensive but it is without effect on normal rats (Muirhead, Leach, Daniels and Hinman, 1968a). Protection against malignant hypertension in rabbits with subcutaneous renal medulla implants only lasts as long as the implants are left in position and electron micrographs show hyperplasia of the renal interstitial cells (RICs) in close proximity to the capillaries (Muirhead, Brooks, Pitcock and Stephenson, 1972).

Working independently of Muirhead, Lee's group identified vasodepressor lipids (VDL) in the rabbit medulla (Lee, Hickler, Saravis and Thorn, 1963) with PGE_2 accounting for the greatest activity (Daniels, Hinman, Leach and Muirhead, 1967).

The Renal Prostaglandins

Prostaglandins have been identified in the renal medullae of all species so far studied including dog (Crowshaw, McGiff, Strand, Lonigro and Terragno, 1970), rat (Somova, 1971), rabbit (Crowshaw, 1971) and man (Vance, Attallah, Prezyna and Lee, 1973), with the highest concentrations in the medulla, the major site of the synthetizing enzyme, whilst the lowest levels are in the cortex, the site of the degrading enzymes (Ånggärd, Larsson and Samuelsson, 1971; Larsson and Ånggärd, 1973). Prostaglandins are believed to be released on synthesis and are not stored in most organs, including the kidney (McGiff and Nasjletti, 1973).

Cultured renal interstitial cells (RIC s) are rich in granules containing triglycerides, fatty acids and prostaglandins E_2, $F_{2\alpha}$ and A_2 (Muirhead, Germain; Leach, Brooks and Stephenson, 1973). According to Janzen and Nugteren (1971) the collecting duct cells provide an even richer source of prostaglandins.

Renal Prostaglandin Synthesis

The common substrate for PGE_2 and $PGF_{2\alpha}$ synthesis is 5,8,11,14 eicosatetranoic acid (arachidonic acid), which is released from phospholipids by acyl hydrolase enzymes such as phospholipase A.

Free arachidonic acid is converted to PGE_2 and $PGF_2\alpha$ via the common endoperoxide intermediates PGG_2 and PGH_2. Non-steroidal anti-inflammatory drugs such as aspirin, indomethacin and meclofenamate prevent the formation of the endoperoxides (see Flower, 1974).

There is great controversy as to whether PGA exists naturally or is a dehydration product of PGE formed on extraction. Combined gas liquid chromatography-mass spectrometry has failed to detect PGA in human blood (Frölich, Sweetman, Carr, Hollifield and Oates, 1975), rabbit renal venous blood (Davis and Horton, 1972), rabbit kidney (Larsson and Ånggård, 1976) and rat kidney (Pugsley, Beilin and Peto, 1975).

However, radioimmunoassay (ria) techniques have detected PGA-like material in human and rat blood (Zusman, 1973; Zusman, Spector, Caldwell, Speroff, Schneider and Mulrow, 1973a; Zusman, Forman, Caldwell, Speroff and Mulrow, 1973b; Lee, 1973; Lee and Attallah, 1975; Hornych, 1976). Less than 2% conversion of PGE to PGA was reported by Zusman, Caldwell and Speroff (1972) during extractions.

To date the only definite evidence of an enzyme capable of forming PGA was reported in human blood and has been named PGA dehydrase since it enzymatically dehydrates PGE (Polet and Levine, 1975).

Prostaglandin Metabolism

15-hydroxy prostaglandin dehydrogenase and prostaglandin 13,14-reductase, present in the cortex, maintain a medullary-cortical prostaglandin concentration gradient (Ånggård et al, 1971). According

to Aiken and Vane (1973) over 80% of the PGE_2 entering the kidney is metabolised and the majority of PGA_1 is degraded to 13,14 dihydro PGA_1 (Attallah, Duchesne and Lee, 1975).

Circulating PGE_2 and $PGF_{2\alpha}$ are removed almost totally by the lungs (Ferriera and Vane, 1967) although PGA passes freely (Horton and Jones, 1969) and this has been verified using ria in dog (Robertson, 1975). The liver is also reported to metabolise PGE (Nakano and Cole, 1969) and PGA (Horton and Jones, 1969).

Renal Prostaglandin Release

Various stimuli are reported to release renal prostaglandins such as renal ischaemia (McGiff, Crowshaw, Terragno, Lonigro, Strand, Williamson, Lee and Ng, 1970a; Herbaczynska-Cedro and Vane, 1973; Gross, Mujovic, Jubiz and Fisher, 1976), renal sympathetic nerve stimulation (Davis and Horton, 1972; Dunham and Zimmerman, 1970), catecholamines (Needleman, Douglas, Jakschuk, Stoeckler and Johnson, 1974; McGiff, Crowshaw, Terragno and Lonigro, 1970b), angiotensin I and II (McGiff, Crowshaw, Terragno and Lonigro, 1970c; Needleman, Kauffmann, Douglas, Johnson and Marshall, 1973; Aiken and Vane, 1973) and bradykinin (McGiff, Terragno, Malik and Lonigro, 1972).

The catecholamine effect has been shown to be due to α receptor stimulation (Needleman et al, 1974) and the competitive angiotensin II antagonist, 8-leu-angiotensin II prevents release due to angiotensin (Needleman et al, 1973).

In isolated rabbit medulla slices only ADH had been shown to cause direct release (Kalisker and Dyer, 1972; Sirvio and Gagnon, 1974) implying that perhaps the prostaglandin release due to other agents is a consequence of haemodynamic or excretion changes. Recently, however, angiotensin has been reported to cause direct release in rat papillary slices (Danon, Chang, Sweetman, Nies and Oates, 1975) and in rabbit papilla (Oates, in personal communication to Zins, 1975).

Intrarenal Role of Prostaglandins

Prostaglandins affect total renal blood flow (RBF), intrarenal blood flow distribution and the diuresis and natriuresis mechanisms.

A. Haemodynamics

PGE_2 infusion (0.01 - 0.30 $\mu g\ kg^{-1} min^{-1}$) into the renal artery of dog increases RBF, whereas $PGF_{2\alpha}$ is without activity (Tannenbaum, Splawinski, Oates and Nies, 1975). In addition PGA_1 is a weak renal vasodilator (Fülgraff and Brandenbusch, 1974). Arachidonic acid elevates RBF in dog and prostaglandin synthetase inhibitors prevent this (Tannenbaum et al, 1975).

In the anaesthetised dog, basal RBF is dependent on a continuous synthesis of prostaglandins, probably of PGE_2 (Lonigro, Itskovitz, Crowshaw and McGiff, 1973), but it is doubtful if there is any dependence in the conscious dog (Zins, 1975; Kirschenbaum and Stein, 1976).

Indomethacin reduces total RBF and cortical blood flow but increases the outer/inner cortical flow ratio in isolated canine kidney (Itskovitz, Stemper, Pacholczyk and McGiff, 1973), and reduced medullary flow is reported in rat (Solez, Fox, Miller and Heptinstall, 1974). Conversely, PGE_2 infusion increases cortical flow with a greater effect on the inner cortex and arachidonic acid increases only inner cortical flow (Chang, Splawinski, Oates and Nies, 1975). A similar finding is reported with arachidonic acid in the rabbit (Larsson and Ånggård, 1974).

Again in the conscious dog prostaglandin synthetase inhibition does not alter intrarenal haemodynamics (Zins, 1975; Kirschenbaum and Stein, 1976), although reduced cortical flow is reported in the conscious rabbit (Beilin and Bhattacharya, 1975).

There is evidence to suggest that renal reactive hyperaemia is mediated by prostaglandins (Herbaczynska-Cedro and Vane, 1974; Owen, Ehrhart, Weidner, Haddy and Scott, 1975; Swain, Heyndrickx, Boettcher and Vatner, 1975). Renal prostaglandins were also implicated as mediating RBF autoregulation (Herbaczynska-Cedro and Vane, 1973) but this has been challenged and will be discussed later in section 3.

B. Excretory Effects of Prostaglandins

1) Natriuresis

The appearance of PG-like material in the renal venous blood after interventions, which lower RBF, is associated with a return of RBF and urine flow (McGiff et al, 1970a, 1970b; Herbaczynska-Cedro and Vane, 1974). PGE_2 and $PGF_{2\alpha}$ produce dose dependent natriuresis in the dog (Fülgraff, Brandenbusch and Heintze, 1974; Tannenbaum et al, 1975) although $PGF_{2\alpha}$ is much weaker and PGA is also a weak natriuretic agent (Fülgraff et al, 1974).

In dog, the natriuresis is accompanied by a fall in urine osmolality due to a rise in free water clearance. Micropuncture studies suggest that PGE_2 inhibits sodium reabsorption in the distal tubule of rat resulting in natriuresis and a rise in urine osmolality (Fülgraff and Meiforth, 1971), whereas in dog PGE_1 but not PGE_2 inhibits proximal sodium reabsorption (Strandhoy, Oft, Schneider, Willis, Beck, Davis and Knox, 1974).

Indomethacin results in a decrease in urine and sodium excretion in anaesthetised dog (Feigen, Klainer, Chaprick and Kadowitz, 1976; Olsen, Magnussen, Eilertsen, 1976) and a similar effect is reported in the rabbit (Lee and Attallah, 1974).

It is therefore generally accepted that the renal prostaglandins mediate natriuresis and may be the long sought after "natriuretic hormone" system. Frusemide is inactive in man (Patak, Mookerjee,

Bentzel, Hysert, Babej and Lee, 1975) and rabbit (Oliw, Kövér, Larsson and Ånggård, 1976) after indomethacin. There are, however, converse findings.

In the conscious dog, prostaglandin synthetase inhibition causes natriuresis, with no change in urine volume flow (Kirschenbaum and Stein, 1976) in agreement with the findings in the isolated dog kidney (Vankerweghem, Ducobu, D'Hollander, 1975).

Human peripheral plasma (Zusman et al, 1973a; Lee and Attallah, 1974) rat plasma and renal PGA_2 concentrations (Zusman et al, 1973b) change inversely with sodium intake as does PGE_2 in the rabbit kidney (Tobian and O'Donnell, 1976). These changes are the opposite expected of a natriuretic agent but it is possible that they reflect changes in response to circulating angiotensin.

Blood volume expansion in the rat raises the blood PG-like activity only if both kidneys are intact (Papanicolou, 1972, 1975) and urinary prostaglandin excretion is also elevated (Papanicolou, Mountokalakis, Safar, Sotiropoulou, Bariety and Milliez, 1976). In support of this, an essential fatty acid deficient diet (Rosenthal, Simone and Silbergleit, 1974), aspirin (Susic and Sparks, 1975) and indomethacin, (Düsing, Melder and Kramer, 1976) prevent the excretion of saline loads in rats. However, arachidonic acid has been reported to hinder saline load excretion in rats (Weber, Holzgreve, Stephan and Herbst, 1975).

In the dog, the injection of sodium chloride into the renal artery causes the release of PGE (Jubiz, Terashima and Anderson, 1976) and acute sodium loading increases the urinary excretion of PGE (Olsen, Magnussen and Eilertsen, 1976) but little is known of the effects of chronic volume loading in this species.

2) Interaction with ADH

There is considerable evidence implicating the renal prostaglandins as antagonists of the renal actions of ADH. PGE_1 inhibits the cyclic AMP dependent increase in water permeability caused by ADH in the toad bladder (Orloff and Handler, 1965) and in the rabbit collecting duct (Grantham and Orloff, 1968). Prostaglandins elevate the free water clearance in dog (Fülgraff et al, 1974; Tannenbaum et al 1975) and in man (Lee, McGiff, Kannegiesser, Mudd, Aykent and Frawley, 1969; Fichman, 1970) but a decrease is reported in the rat (Fülgraff and Meiforth, 1971).

In the hypophysectomised anaesthetised dog, the antidiuretic effect of exogenous ADH is potentiated by indomethacin or meclofenamate pretreatment (Anderson, Berl, McDonald and Schrier, 1975a). The diuresis seen with clonidine in the dog is associated with a rise in urine PGE-like activity with no change in ADH excretion. Both ADH and indomethacin prevent the clonidine induced diuresis (Olsen, 1976).

ADH is one of the few substances which directly releases PGE from the kidney (Kalisker and Dyer, 1972; Sirvio and Gagnon, 1974) and in view of the evidence above showing that PGE can antagonise the action of ADH on the collecting ducts, then the case for renal prostaglandins acting as physiological modulators of the ADH antidiuresis is strengthened.

Curiously in the isolated canine kidney, devoid of ADH, indomethacin increases collecting duct permeability, suggesting that either indomethacin acts directly on the collecting ducts or else the renal prostaglandins can act directly on the ducts to decrease permeability (Vankerweghem et al, 1975).

3) Prostaglandins - Renin Interactions

Renal prostaglandins may affect the renin system in several ways.

a) The intrarenal vasoconstrictor effect of angiotensin II is antagonised by the renal prostaglandins since inhibition of prostaglandin synthesis leads to potentiation of the renal vasoconstriction (Aiken and Vane, 1973; Satoh and Zimmerman, 1975a). Angiotensin releases renal prostaglandins (McGiff et al, 1970a, 1970b; Needleman et al, 1973; Aiken and Vane, 1973), possibly by a direct mechanism (Danon et al, 1975). The acute rise in angiotensin concentration during unilateral renal artery constriction in the dog leads to a reduction in renal blood flow of the contralateral kidney (Satoh and

Zimmerman, 1975b) and releases prostaglandins from this kidney (McGiff et al, 1970a; Pamnani, Simon and Ovenbeck, 1976).

The ischaemic kidney in dog is also reported to release prostaglandins (McGiff et al, 1970a) and this appears to be due to the renin released since angiotensin antagonists prevent the prostaglandin release (Satoh and Zimmerman, 1975a).

b) Early reports conflicted as to how prostaglandins affect renin release probably due to the use of infusions, which may not mimic the effects of endogenous release (see Larsson, Weber and Ånggård, 1974).

In the rabbit sub-hypotensive doses of arachidonic acid elevate and indomethacin lowers PRA (Larsson et al, 1974). Indomethacin produces a similar effect in volume loaded rats (Weber et al, 1975). The rise in PRA after bilateral renal artery stenosis in the rabbit, is prevented by indomethacin (Ånggård, Larsson and Weber, 1976). In man PRA is decreased by indomethacin (Rumpf, Frenzel, Lowitz and Scheler, 1975) whilst PGA_1 does not affect PRA but causes direct release of aldosterone (Fichman and Horton, 1973).

c) PGA_1 and A_2 but not the E series are competitive inhibitors of human renin (Kotcher and Miller, 1974), which may be of relevance in vivo, since bilateral nephrectomy in rats has

been shown to remove an inhibitory influence upon the renin kinetics (Romero, Lazar, Elkins and Hoobler, 1969). In the two-kidney Goldblatt rabbit, removal of the untouched kidney also removes the renin inhibitory factor (Lazar, Romero and Hoobler, 1971). The hypertension in dog, during the first two hours after clamping has been shown to be renin-dependent and the hypertension is milder in the two-kidney Goldblatt dog than in the one-kidney preparation, for a given PRA (Bianchi, Baldoli, Lucca and Barbin, 1972).

These studies suggest the presence of a renal renin inhibitor, not necessarily a prostaglandin, but possibly the lysophospholipid described by Sen, Smeby and Bumpus (1968). Osmond (1972) reported an elevation of this inhibitor in the contralateral kidney of the two-kidney Goldblatt rat.

Whilst a renin preinhibitor may play a role in the control of BP it cannot explain the antihypertensive effect of renal medulla in anephric animals (Muirhead et al, 1960) or in the later stages of hypertension when renin levels are normal (see discussion of section 1).

Prostaglandin - Sympathetic Interactions

Prostaglandins modulate adrenergic activity by acting at pre- and post-junctional sites of the adrenergic nerve. PGE_2 and arachidonic acid decrease noradrenaline overflow from the rabbit kidney during nerve stimulation (Frame and Hedqvist, 1975). PGE_1, E_2 and A_2 attenuate the vasoconstriction caused by nerve stimulation in the rabbit kidney

but do not affect the response to noradrenaline and $PFG_2\alpha$ potentiates both stimuli (Malik and McGiff, 1975). A similar response is reported in the dog kidney, with the exception that the noradrenaline response is also slightly attenuated (McGiff et al, 1970b). As noradrenaline (Needleman et al, 1974; McGiff et al, 1970b) and renal nerve stimulation (Dunham and Zimmerman, 1970; Davis and Horton, 1972) both release renal prostaglandins, then it is proposed that the renal prostaglandins may act as modulators of intrarenal sympathetic activity.

Curiously in the rat kidney, arachidonic acid, PGE, PGE_2 and PGA_2 potentiate nerve stimulation and noradrenaline and at higher doses cause direct vasoconstriction (Malik and McGiff, 1975).

Extra-renal Effects of Prostaglandins

In dog PGE and PGA are vasodilators, A being more active by the intravenous route although less active intra-arterially due to the pulmonary degradation of PGE (Horton and Jones, 1969). These prostaglandins also dilate dog hind limb capacitance vessels (Conway and Hatton, 1975) as does PGC_2 (Jones, Kane and Ungar, 1974) and is in fact more active than either PGA_2 or PGE_2 (unpublished observations, 1973).

In the dog and rat, $PGF_2\alpha$ is a pressor agent due to venoconstriction and elevation of the cardiac output (Ducharme, Weeks and Montgomery, 1968). $PGF_2\alpha$ has been shown to potentiate adrenergic venoconstriction (Kadowitz, Sweet and Brody, 1972) and PGD_2 and PGC_2 potentiate catecholamine induced venoconstriction, although to a lesser degree than $PGF_2\alpha$ (Jeffrey and Smith, 1977).

A rise in peripheral resistance (Nakano and Cole, 1969) and central effects (Sweet, Kadowitz and Brody, 1971; Lavery, Lowe and Scroop, 1971) have also been implicated in the pressor action of $PGF_{2}\alpha$.

According to Jones (1976) there are at least two receptor types in the vascular system responsible for prostaglandin effects, namely the depressor effects due to E-like prostaglandins and pressor responses due to D-like prostaglandins (i.e. PGA, PGC and $PGF_{2}\alpha$ respectively).

One problem in accepting an extrarenal role for renal prostaglandins is the pulmonary degradation of PGE and PGF (Vane and Ferriera, 1967; Robertson, 1975). PGA (Horton and Jones, 1969; Robertson, 1975), PGC (Jones, 1972) and PGB (Robertson, 1975) all escape pulmonary degradation. In the rat, unlike the dog, PGE is less efficiently removed by the lungs (Papanicolou and Meyer, 1972).

Not all the pulmonary degradation products are without biological action, however. In sheep, 15-oxo $PGF_{2}\alpha$ is 5 to 10 times more active at raising BP than $PGF_{2}\alpha$, whereas 15-oxo PGE_{2} is inactive (Jones, 1975). 15-oxo PGA_{2} is inactive in rat, but the renal metabolite of PGA_{1} 13,14 dihydro PGA_{2} is as active at reducing BP as PGA_{2} (Lee, 1976).

Prostaglandins in Experimental Hypertension

Rat kidney (Tobian et al, 1964) and cultured RICs (Muirhead et al, 1973) are antihypertensive in the rat. The medulla from the ischaemic kidney is more potent than normal medulla and that from the untouched kidney is without activity (Manthorpe, 1973).

There is evidence that renal prostaglandins do not mediate this action in this species. Indomethacin treatment fails to prevent the antihypertensive effect of implanted normal medulla (Manthorpe, 1975) and the lipids extracted from rat RICs incubated with indomethacin are still antihypertensive (Muirhead, Leach, Germain, Byers and Armstrong, 1974).

There are conflicting reports regarding renal levels of prostaglandins in hypertensive rats. A rise in PGE is reported in the clipped kidney (Somova, 1971; Jaffe, Parker, Marshall and Needleman, 1972).

In contrast a decreased synthetic capacity is reported in one and two kidney Goldblatt rat and in the Japanese SHR, with a good correlation with the degree of hypertension (Sirvio and Gagnon, 1974). Decreased release is reported in the isolated kidney of Goldblatt rat (Leary, Ledingham and Vane, 1974).

Prostaglandin synthetase inhibition produces hypertension in rats but only after volume loading (Rosenthal et al, 1974) and renal hypertension is exacerbated by indomethacin (Pugsley, Beilin and Peto, 1976) and a variety of aspirin-like drugs (Schölkens and Steinbach, 1975). However, meclofenamate has been reported to produce a fall in BP during the acute phase of renal hypertension in rats (McQueen and Bell, 1976).

PGE_1, E_2, A_1 and A_2 are antihypertensive in the rat when injected daily (i.p.) (Somova, 1972) although other workers claim higher doses of PGE are required (Muirhead, Leach, Brown, Daniels and Hinman, 1967; Wendling, Ducharme and Graham, 1972; McQueen and Bell, 1976). A central action is also reported with PGA_2 in the SHR (Leach, Armstrong, Germain and Muirhead, 1973).

The first report of hypertension after chronic treatment with indomethacin was reported in the rabbit (Colina-Chourio, McGiff and Nasjletti, 1975), however Muirhead, Brooks and Brosius (1976), failed to repeat this.

In the dog the status of the renal prostaglandins in experimental renal hypertension has been poorly documented despite the fact that this species probably offers the best example of the antihypertensive role of the kidney.

Acute reduction of RBF has been reported to release prostaglandins (McGiff et al, 1970a; Herbazynska-Cedro and Vane, 1973) but this may be a misinterpretation of the data (Beckman and Zehr, 1975) as discussed in section 3.

During the acute phase of renal hypertension in the dog there is a rise in output of prostaglandins from the untouched kidney (McGiff et al, 1970a) probably due to the rise in angiotensin.

Oral PGE_2 given to one kidney Goldblatt dogs daily reduces BP (Muirhead, Leach, Brooks, Brown, Daniels and Hinman, 1968b). In the anaesthetised dog, prostaglandin synthetase inhibition raises BP (Lonigro et al, 1973a).

The absence of reports of renal prostaglandin studies in conscious renal hypertensive dogs prompted the present investigation.

SECTION I

RENAL VENOUS PROSTAGLANDINS IN EXPERIMENTAL RENAL HYPERTENSION IN DOG

Problems Encountered in Chronic Dog Work

In the course of this investigation, having had no previous experience with conscious catheterised dogs, we encountered several problems, which are discussed below.

Catheter Patency

The most noticeable early problem was that of catheter patency. In the early preparations we used a skin button arrangement, containing a valve as a means of exteriorizing the renal and jugular venous catheter. The design was that of Day and Whiting (1972). In our hands, the valves occasionally leaked causing catheter blockage. We eventually resorted to using metal Luer fittings with metal obturators.

Catheter block still proved a problem, but this was further reduced by using PVC carotid and jugular catheters instead of nylon and the use of silastic narrow bore tubing as the renal venous catheter. Clotting within the carotid artery catheter was virtually eliminated by ensuring that the catheter tip lay in the aortic arch and not in the origin of the carotid artery, where the "dead" portion exists.

Having reduced the incidence of catheter blockage, the frequency of catheters being pulled out rose and this was reduced by the use of a subcutaneous collar around the catheter and by securing the catheters externally in an elastic bandage placed around the neck of the dog.

Haemorrhage from the blood vessel, at the site of catheterisation was prevented by double ligation distal to the point of entry.

Infections

Systemic and local infections were particularly frequent in the early experiments, manifesting themselves in the first 2-3 days after the operation. On the advice of the Bacteriology Department, the use of ampicillin as a post-operative prophylactic was abandoned and thiomersal was included as an antiseptic in our surgical routine, due to the high incidence of Pseudomonas pyocyanea infections. Post-operative infections were consequently reduced in incidence and only occasionally did sytemic infections arise, presumably due to bacteria introduced during daily catheter flushing and during fluid volume estimations. Benzylpenicillin with streptomycin (Cristamycin, Glaxo) normally controlled such infections.

Post-operative Recovery

Poor abdominal wound healing tended to hinder the post-operative recovery of some dogs and this was solved by changing from a median to a para-median abdominal incision, which although technically more difficult due to musculature and vasculature, resulted in a mechanically stronger wound.

Animal Health and Size

General improvements in the preparations were also seen when more rigorous attention was paid to the dogs' general health. Larger dogs endured the operation better than the initial small ones and eventually a source of reasonably healthy large foxhound bitches was found. These were normally kept in the animal house for at least 2 weeks prior to the operation and were fed on a standard diet

with added ferrous sulphate.

The first completely successful dog, Sadie, was operated on November 1975. Thereafter all the foxhounds providing successful results, weighed between 16.5 and 24.5 kg (20.3 $\pm$ 2.7 kg, mean $\pm$ sem, n = 12).

METHODS

Surgical Procedures

Pre-operative procedures

On the morning of the operation, the dog was pre-medicated with pethidine (Pethilorfan, Roche, 50 mg i.m.). After 30 min, the dog was taken to the preparation room, where the dorsal midline was shaven, such that the dorsal midline could be marked as a guide to catheter exteriorization during the operation. The dog was anaesthetised with pentobarbitone (Nembutal, Abbott, 30 mg/kg, i.v.) and the flanks, neck and abdomen clipped and the loose hair removed with a vacuum cleaner. The shaved skin was swabbed with 3 antiseptics. The first antiseptic used was Stericol (Sterling Industrial) and this was followed with alcoholic chlorhexidine (Hibitane, I.C.I.). Immediately before incisions in the theatre, thiomersal solution (0.1% w/v thiomersal in 50% ethanol, 25% acetone and 25% water) was used.

Surgical procedures

The animal was carried into the operating theatre and secured on its back to the operating table. A heating pad was placed under the animal to maintain its temperature. A Venocath (Abbott) was inserted into a saphenous vein for the infusion of Hartmann solution (Steriflex No. 11, Allen and Hanburys Ltd) to maintain acid-base balance and to replace fluid loss during the operation. Supplementary doses of pentobarbitone were also administered by this route.

Those participating in the sterile activities of the operation scrubbed up and put on sterile masks, caps, gowns and gloves. The abdominal site was draped, swabbed with thiomersal and the first incision, a paramedian ventral incision was made with diathermy. This was preferred to a ventral midline incision because of the greater strength and healing ability. The muscle layers were split with blunt dissection and diathermy. Bleeding was stopped by ligation or coagulation. The peritoneum was opened and the gut exteriorized to expose the right renal region. The peritoneum above the right renal artery was cut and the artery freed for about 1 cm. The renal clamp was introduced through a stab-wound at the subcostal angle and was fitted around the renal artery. The silastic tube leading to the clamp (1.6 mm i.d., 5.0 mm o.d.) was cut to size (60 - 100 mm in length) and buried subcutaneously with a stopper and a loop of thread to allow it to be retrieved at the time of clamping. The stab-wound was stitched and the renal peritoneum repaired using continuous stitches (3 metric, ivory thread, Mersilk, Ethicon).

The peritoneum above the ovarian vein on the left side was incised and the vein dissected free for about 2 - 3 cm to its junction with the renal vein. The silastic renal venous catheter (Esco Ltd, 1.0 mm i.d., 2.0 mm o.d. and about 70 - 75 cm long, with side holes in the last centimetre) was introduced into the abdomen through a stab-wound from a track down the animal's back. Two collars were placed around the catheter 3 cm from the tip to prevent the catheter from being pushed or pulled from the required position. The catheter was positioned such that the tip could be palpated in the renal vein. The peritoneum was then repaired.

In one dog (Sadie), the left renal artery was clamped such that the sampling was from the clamped kidney (ipsilateral samples).

The ventral peritoneum was repaired using continuous stitches and the muscle layers were drawn together with individual gut stitches (4 metric, Chromic, Ethicon). Subcutaneous gut stitches were also used and the skin was sutured with black silk (4 metric, Mersilk, Ethicon).

On completion, the wound was irrigated with thiomersal and sprayed with polymixin, neomycin and bacitracin (Polybactrin, BDH) and was finally covered with a film of Nobecutane (BDH) and gauze.

The left common carotid artery and the right external jugular vein were exposed after a midline ventral incision in the neck using diathermy, followed by blunt dissection of the muscle. The arterial and venous catheters (PVC, 30 - 35 cm long, 2.0 mm i.d., 3.0 mm o.d., with side holes in the venous catheter) were introduced at the back of the neck, through the muscle and inserted into the vessels, such that the tips were judged to be in the aortic arch and in the vena cava respectively. The arterial, central venous and renal venous catheters were all led to the back of the neck and terminated with metal luer fittings (BDB Products).

A mercury manometer was used to ensure that the arterial catheter had not entered the left ventricle (mean pressure of about 70 mm Hg).

The neck was stitched and a tubular elastic bandage (Tubigrip, Seton Products Ltd) was doubled and placed around the dog's neck. The catheters were pulled through the inner layer and secured to the

bandage. Their dead spaces (less than 1 ml) were cleared of the weak heparin solution (100 μ/ml) and refilled with 1000 μ/ml of heparinised saline.

Post-operative care

On completion of surgery, the animal was removed to its kennel and laid on an electric blanket to recover. The operation was normally completed by 14.00 hours, taking less than 3 hours to perform. By 17.00 hours the animal had usually recovered consciousness. Solid food was witheld for 24 hours, only water and milk with Complan were allowed. Every morning, the dead spaces of the catheters were removed and the catheters were flushed with sterile saline (10 ml total each day) and the dead spaces refilled with 1000 u/ml heparinised saline. On rare occasions the catheters blocked and streptokinase (Kabikinase, Kabii) was injected or a sterile catheter wire was pushed down.

Rectal temperature was taken daily and a progress chart kept. After signs of infection, swabs were taken from any localising site for a bacteriological report. Meanwhile 500 mg streptomycin and 300 mg benzyl penicillin (Cristamycin, Glaxo) was given (i.m.) twice daily. Fortunately all cases of infection responded to this regime.

The dogs were allowed one week to recover from the operation and they were placed on a convalescent diet (Pedigree Pet Foods Ltd) with a constant sodium content of 32 mEq/can. For a typical 20 kg dog on two cans per day, this meant a solid food sodium intake of about 3.2 mEq $kg^{-1}day^{-1}$.

Experimental Procedures

Control period

On the second week after the operation, the control parameters were measured. The animals had been introduced to the laboratory environment several times before the operation. At about 10.00 hours, the dog was placed in a large basket and allowed to lie. The carotid and central venous catheters were flushed and connected to Consolidated Electrodynamic L223 transducers and the mean and pulstatile pressures were recorded on magnetic tape (Bell and Howell, 7 channels, VR3200) for 3 minutes after the animal had settled.

In addition to BP and CVP, plasma volume (PV) and extracellular fluid volume (ECFV) were also measured. All these control recordings were made at least twice.

Arterial and renal venous blood samples were also taken for prostaglandin (10 ml/sample) and renin (5 ml/sample) estimations.

Clamping operation

At the beginning of the third post-operative week, the animal was made hypertensive by partially clamping the renal artery. Under thiopentone (Pentothal, Abbott) anaesthesia, the clamp tubing was located under a cut-down and the long blade screwdriver inserted down the tube to locate the screw. The screw was turned to occlude the artery completely and then screwed back 1½ turns (0.8 mm). This normally produced a rise in BP within a few seconds.

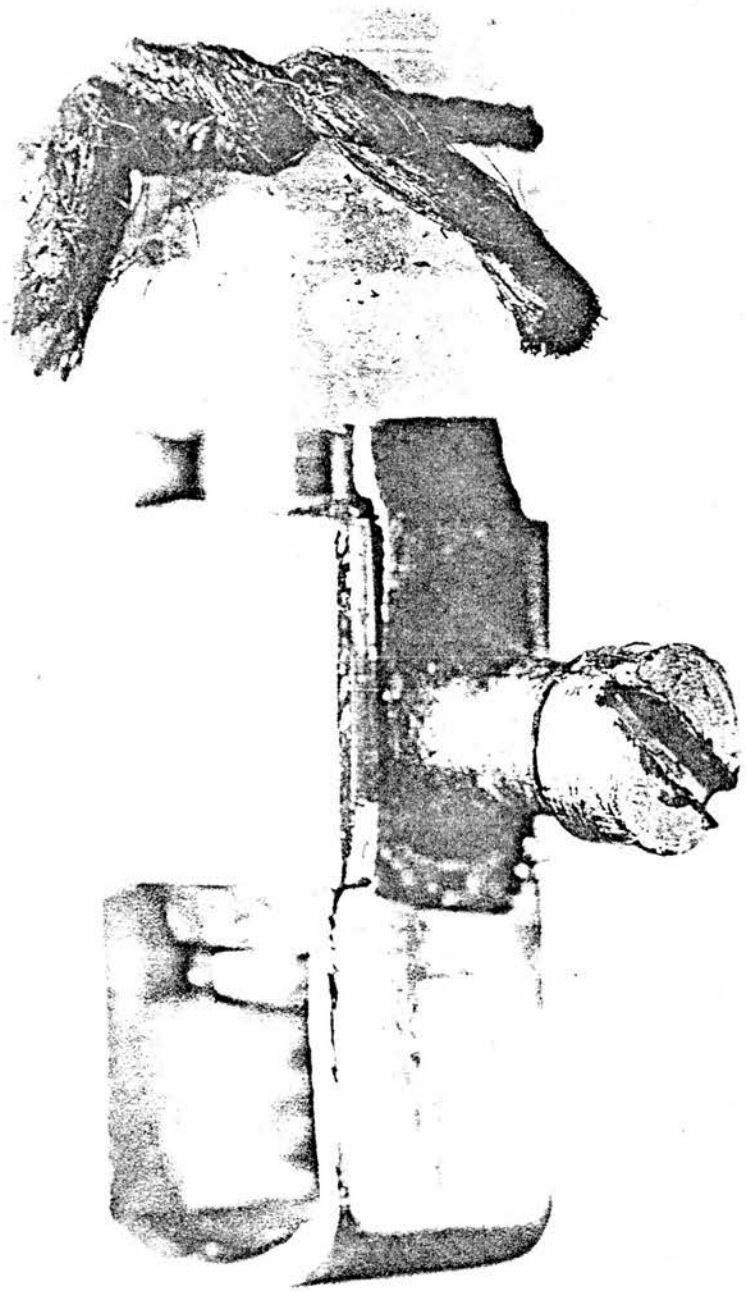

Figure 1 Renal artery clamp showing locking screw on the right with the vertically moving occluding screw within the 'U'-shaped tail-piece. The scale on the right is in millimetres.

The clamp used in this study was a modification of that designed by Ferrario, Blumle, Nadzam and McCubbin (1971). Essentially it consisted of a head-piece with a small screw, which could be screwed down to compress the artery, which lay in the 'U'-shaped tail-piece. The clamp was modified by a locking screw, since in early studies it was found that the clamp could spring apart if the compressing screw was lowered. The clamp was made from high quality surgical steel by Mr G Campbell of the departmental workshop (see Figure 1).

In some of the early dogs, intravenous pyelograms (ivp) were performed to gain some indication as to the extent of clamping as judged by preservation of renal function. Figure 2 shows the ivp of Melody 3 days after clamping. Bilateral function was present.

Hypertension period

During the next 2 to 3 weeks, the hypertension was monitored by measuring BP, CVP, PV, ECFV, haematocrit (Ht), plasma sodium and potassium, renin and prostaglandins at regular intervals.

Blood collection

10 ml blood samples from the arterial and renal venous catheters were used for prostaglandin estimations. These samples were immediately placed in 10 ml conical centrifuge tubes, containing heparin and surrounded with ice.

5 ml blood samples were used for renin estimation and these were placed in tubes containing approximately 4 mg EDTA (disodium salt).

POST CLAMP I.V.P.

Figure 2a

X-ray of Melody, 2 days after clamping the right renal artery. The outline of the clamp and its locking screw are clearly visible.

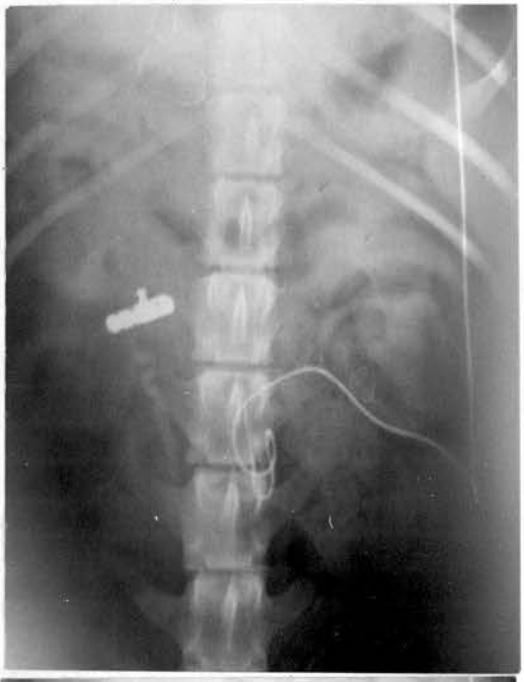

Figure 2b

2 minutes after injection of the radio-opaque material into the left renal venous catheter. The material is present in the full length of the right ureter and is beginning to fill the left ureter.

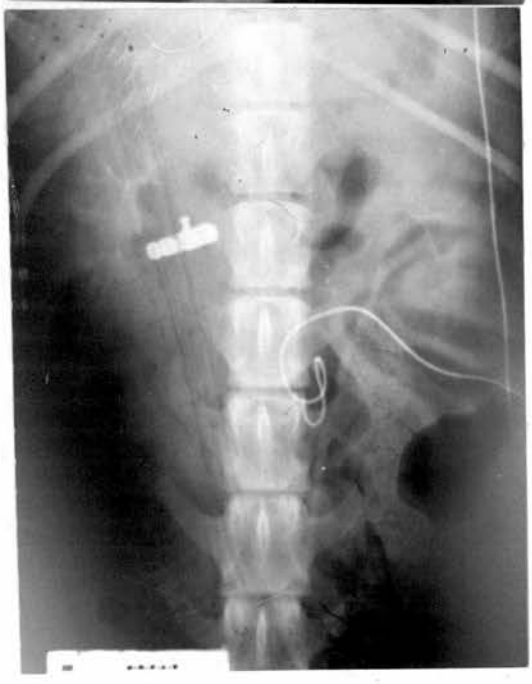

Figure 2c

6 minutes after injection, the full length of the left ureter is visible and the right renal calyces are now visible. The bladder can be faintly seen.

The blood was centrifuged as soon as possible at 4°C, 15,000 g for 25 min and the plasma decanted and placed in labelled blood tubes, which were stored in a freezer until assay.

Haemodynamic measurements

The BP and CVP signals were played back on the tape recorder and displayed permanently on light sensitive paper (Honeywell, Visicorder, 2206).

Heart rate (HR) was computed by a programme patched on an analogue computer (EAL 380). The positive first differential of the BP pulse was used to trigger a time ramp, which was reset and immediately set in the integrate mode by the next rise in BP. The peak voltage of the time ramp was stored by 2 track/store amplifiers in series and this value (heart period), was fed into a divider unit to obtain heart rate on a beat by beat basis. The system was calibrated by use of the mode timer set to 500 + 500 msec (60 beats/min) and 200 + 300 msec (120 beats/min). HR was also recorded on the light sensitive paper.

Fluid volume estimations

PV and ECFV were measured by estimating the dilution of injected material.

A control sample of arterial blood (5 ml) was taken and the haematocrit estimated using microhaematocrit tubes. After centrifuging, 0.5 ml plasma was used for estimation of control levels for the PV estimation

and a further 0.5 ml for the ECFV control estimation. 1.0 ml was used for plasma electrolyte estimation.

After injection of the tissue fluid markers, 2 x 1.5 ml blood samples were removed after 10 and 90 min for PV and ECFV estimation respectively (0.5 ml plasma in each case).

A total of 8 ml blood was used for each series of fluid volume measurements. The exact methods for these measurements are as follows:-

Plasma volume (PV) estimation

T-1824 (Evans Blue) is an azo dye, which binds to plasma proteins and leaves the vascular compartment at a very slow rate (Gibson and Evans, 1937). It is possible to estimate plasma volume (PV) from the dilution of a known amount of injected T-1824. Since the rate of disappearance is slow and equilibrium occurs within three minutes (Surtsin and Rolf, 1950) a blood sample taken 10 minutes after injection can be used to estimate PV (Bianchi, Tenconi and Lucca, 1970; Ferrario, 1974).

Haemolysis and lipaemia interfere with the spectrophotometric determination of T-1824 and several workers employ extraction techniques or use several wavelengths to correct for interference. In the present experiments T-1824 was still detectable from previous tests and as a result plasma samples were taken before each test to correct for circulating T-1824. This also corrected for any lipaemia. Haemolysis was found to cause negligible interference despite large absorption peaks at 415, 545 and 580 nm, characteristic of haemoglobin.

The T-1824 was made up in batches by dissolving the powder (Gurr) in sterile 0.9% saline (Steriflex No. 1, Allen and Hanburys Ltd) to give a final concentration of 4 mg ml^{-1}. The solution was filtered through a Millipore filter (microsyringe filter with a 0.22 μ filter) into ampoules, which were sealed for future use.

6 ml (24 mg) was injected into the jugular vein catheter and washed in with saline after a control arterial sample had been taken. After 10 minutes, an arterial sample (1.5 ml) was withdrawn and the plasma removed after centrifugation for T-1824 estimation. Haematocrit was measured using microhaematocrit tubes (MSE).

In view of the fact that T-1824 slowly degrades in light and to avoid errors in making fresh standards each time, the injectate solution was used to make three standard solutions. 0.5 ml of injectate was dissolved in saline (50 ml) for the preparation of solutions equal to 200, 400 and 800 times the dilution of the injectate as shown in Table 1.

T-1824 in plasma has an absorption peak at 625 nm but in saline the OD at 625 is lower for a given concentration and the peak absorption is at 605 nm and is pH dependent. In phosphate buffer (pH 7.4) peak absorption is also at 605 nm but the reading at 625 nm is equivalent to that in plasma. Therefore the standards, dissolved in phosphate buffer, were used to make a calibration.

Table 1: T-1824 Estimation

	Control	Test	Standard Dilutions			Blank
			200x	400x	800x	
Plasma	0.5	0.5				
Phosphate Buffer	1.5	1.5	1.0	1.5	1.75	2.0
Diluted Injectate			1.0	0.5	0.25	

Volumes in ml

The reciprocal of the dilution factors 200, 400 and 800 times, 5×10^{-3}, 2.5×10^{-3} and 1.25×10^{-3} plotted against the OD at 625 nm is linear and the plasma dilution factor can be read from this graph and PV estimated from:-

$$PV = \frac{\text{injectate volume (ml)} \times \text{dilution factor}}{4}$$

and blood volume (BV) = $\frac{PV}{1-Ht}$

Extracellular fluid volume (ECFV) estimation

ECFV was estimated by measuring the extent of dilution of potassium thiocyanate (KSCN) as first described by Ashworth, Muirhead, Thomas and Hill (1943).

The thiocyanate anion distributes itself fairly evenly over the first 4 hours and is excreted at about 2.3% per hour. To avoid blood loss, rather than take several samples and extrapolate to zero time, a 90 minute sample was taken (Bianchi, Tenconi and Lucca, 1970). This method tends to overestimate ECFV because KSCN enters the cells of the gastric mucosa and erythrocytes (Ashworth et al, 1943) but it can nevertheless be used as an index of ECFV changes (Levitt and Gaudino, 1950).

Thiocyanate (BDH, Analar) was made in batches by dissolving the powder in 0.9% saline to give a final concentration of 50 mg ml^{-1}. The solution was passed through a Millipore filter and ampouled.

A control arterial blood sample was used to correct for endogenous levels and circulating thiocyanate from previous tests. 10 ml of thiocyanate (about 25 mg kg^{-1}) solution was injected into the jugular vein catheter over one minute. Rapid injection resulted in cardiac arrest in one dog because the thiocyanate ion is a powerful stimulant of cardiac tissue. After 90 minutes an arterial blood sample (1.5 ml) was taken and centrifuged and the plasma analysed for thiocyanate after the method of Elkington and Taffel (1942).

Plasma protein of both the control and 90 minute samples was precipitated using 2 ml of 10% trichloro-acetic acid, whilst vortexing on a 'Whirlimix'. After centrifugation, the supernatant was removed and reacted with 2.5% (w/v) ferric nitrate in 10% nitric acid, resulting in an orange coloured solution. These were compared with standard solutions by reading the absorption at 460 nm in a spectrophotometer (Bausch and Lomb, SP 20).

The standard stock solution of 15 µg ml^{-1} was used to give standards of 2.5, 5 and 10 µg ml^{-1}. The injectate was diluted 1:2500 (0.1 ml in 250 ml) and assayed in triplicate to obtain an accurate estimation of the concentration injected. The control and 90 minute samples were measured in duplicate. See Table 2.

Immediately after adding the ferric nitrate reagent, the tubes were mixed and read at 460 nm. ECFV was calculated from:-

$$\text{ECFV} = \frac{\text{injected volume (ml)} \times \text{injectate conc. (mg ml}^{-1}\text{)}}{\text{plasma 90 min. conc. - 0 min. conc. (mg ml}^{-1}\text{)}}$$

Table 2: Thiocyanate Estimation

	Control	Test	Standards (µg/ml)			Injectate	Blank
			2.5	5.0	10.0		
Protein-free plasma	1.0	1.0					
15 ug/ml standard			0.5	1.0	2.0		
1:2500 injectate						1.0	
Distilled water	1.0	1.0	1.5	1.0		1.0	1.0
10% TCA							1.0
Ferricyanide reagent	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Volumes in ml

Interstitial fluid volume (IFV) was calculated from the difference of ECFV and PV and the ratio PV/IFV was used as an index of fluid distribution between the vascular and the extravascular compartments. An abnormal distribution is reported in Goldblatt rat (Lucas and Floyer, 1974) and in hypertensive man (Tarazi, Dustan and Frohlich, 1969).

Plasma sodium and potassium concentrations

1.0 ml of plasma was diluted to 50 ml by dissolving in 49 ml of distilled water for K^+ estimation and 2 ml of this was diluted to 10 ml for Na^+ estimation using a flame photometer (EEL).

The sodium standards were prepared from a stock solution of sodium chloride (100 mEq litre^{-1}) by making 0.4, 0.5 and 0.6 ml of this solution up to 100 ml. This produced solutions of 0.4, 0.5 and 0.6 mEq litre^{-1} equivalent to plasma Na^+ concentrations of 100, 125 and 150 mEq litre^{-1} respectively.

Since potassium interferes with the sodium estimation (the reverse is not true), 0.2 ml of 10 mEq litre^{-1} KCl was added to each standard. This is equivalent to a plasma concentration of 5 mEq litre^{-1} K^+.

The K^+ standards were prepared from a stock solution of 10 mEq litre^{-1} KCl by dilution of 0.6, 0.9 and 1.2 ml of 100 ml to give solutions of 0.06, 0.09 and 0.12 mEq l^{-1}, equivalent to plasma concentrations of 3.0, 4.5 and 6.0 mEq litre^{-1} respectively.

These standards gave a linear calibration, from which the plasma concentrations could be calculated.

Prostaglandin Radioimmunoassay (RIA)

Prostaglandin E_2 and $F_2\alpha$ and B_2 antibodies were raised in rabbits and PGA_2 antibody was raised in sheep. The most suitable antisera were used for the radioimmunoassay of prostaglandins extracted from the dog blood samples.

Production of Antibodies

A. Conjugate preparation

PGE_2 was conjugated to bovine thyroglobulin (Tg) using the carbodiimide reaction. After 4 hr of incubation at room temperature, the mixture was dialysed in phosphate buffer for 10 hr, against running water. After dialysis, the retentate was centrifuged for 20 min at 40°C and 15,000 g and the supernatant was freeze-dried to yield PGE_2-Tg conjugate. Each mole of thyroglobulin bound 334 moles of PGE_2. PGA_2 and PGB_2 were also conjugated to Tg.

$PGF_2\alpha$ was conjugated to bovine serum albumin (BSA) by the mixed anhydride reaction. After incubation at about 0°C for 4 hr, the solution was dialysed for 48 hr against running water. The conjugate obtained had about 20 - 37 moles of $PGF_2\alpha$ bound to each mole of BSA.

B. Injection regimen

White New Zealand rabbits were injected intradermally, on the shaved back, at about 50 sites with a total of 100 µg conjugate in 2 ml of Freund's complete adjuvant. Booster injections were given every 6 weeks at 4 sites (suprascapular and femoral) using about 250 µg/site.

Three sheep were used to raise the PGA antibodies and these were injected subcutaneously on the back at regular intervals.

C. Blood collection

The rabbits were bled at regular intervals after the first boost, from the marginal ear vein, to provide about 50 ml of blood each time. The blood was allowed to clot at room temperature and then left overnight at $4^{o}C$. The serum was centrifuged at 1,000 g for 2 min at $4^{o}C$ and then decomplemented at $56^{o}C$ for 30 min. Sodium azide (0.1 ml of a 10 mg/ml solution to every 10 ml of serum) was added and the serum was stored in sterile McCartney bottles, in aliquots of 2 ml, at $-20^{o}C$. The sheep were regularly bled from an external jugular vein and the blood treated in the same way.

Extraction Procedure

500 µl of plasma was routinely assayed, but in some cases when levels were low, the assay was repeated using 1 ml. The plasma was acidified to a pH of 5.0 with 1.0 MHCl (approximately 20-40 µl). The addition of petroleum ether at this stage to remove the neutral lipids does not improve the assay and so was omitted in the extraction procedure. The prostaglandins were extracted twice in 6 volumes of ethyl acetate and the combined ethyl acetate fractions were washed with 500 ul of distilled water and evaporated to dryness. The dry extract was dissolved in 400 µl of 60:40:20 toluene/ethyl acetate/methanol and diluted with 600 µl of 60:40:0 and left overnight at $-20^{o}C$. The extraction recoveries of added ^{3}H-prostaglandins were 56 $\pm$ 13 for PGA_2, 78 $\pm$ 11 for PGE_2, 86 $\pm$ 9 for $PFG_2\alpha$ and 85 $\pm$ 12 for PGB_2 (mean $\pm$ s.d., n = 12).

Chromatographic Separation

Small columns (Pasteur micropipettes, 100 x 5 mm) were filled with 0.5 gm slurry of silicic acid (Sigma, 100-300 mesh) in 60:40:0. Each column was pre-washed with 5 ml of 60:40:20 and 2 ml of 60:40:0 and the extracted prostaglandins were added to the columns and flushed twice with 250 µl of 60:40:0.

The following fractions were obtained:-

Fraction 1 - eluted with 6 ml 60:40:0.5, consisting of the A and B prostaglandins.

Fraction 2 - eluted with 12 ml 60:40:1, consisting of E metabolites.

Fraction 3 - eluted with 6 ml of 60:40:3, consisting of PGE.

Fraction 4 - eluted with 4 ml 60:40:20, consisting of PGF.

Fractions 1, 3 and 4 were evaporated to dryness, ready for radioimmunoassay.

Column recoveries were between 40 and 65%.

Radioimmunoassay Procedures

A. Dilution Curves

The antisera was diluted in a series of 2-fold dilutions using diluent (0.05 M tris buffer with a pH of 6.8 for the PGE antibody and 7.3 for the PGF antibody). To each tube containing 1 ml of antiserum, 50 µl of tracer solution (20 pg PGE_2 and $PGF_{2\alpha}$, 120,000 - 170,000 mCi/mmol, Hamersham) was added and the tubes were incubated

for 2 hr at 4°C. After incubation, 50 µl of diluted normal rabbit antiserum was added and the solution left to equilibrate for 10 min. 50 µl of donkey rabbit antiserum (DARS, Wellcome Reagents, 3 in 10 dilution) was added and after 16 hr of incubation at 4°C, the tubes were centrifuged at 4°C for 45 min at 1500 g. 0.6 ml of supernatant was transferred to a vial of scintillant (PPO and DMPOPPOP, 13 ml) and counted for 4 or 10 min on a Nuclear Chicago liquid scintillation counter, with a counting efficiency of about 30%. The PGB antibody was treated similarly to the PGE and PGF antibodies. PGA antibody was also similarly treated, with the substitution of donkey anti-sheep serum (DASS) for the DARS.

A final dilution curve was chosen, which gave a binding of about 60% of the tracer in the absence of "cold" standard.

B. Standard Curves

Duplicate standard tubes were used in the range 10 pg to 5.1 ng of unlabelled prostaglandin standard made up to a final volume of 0.75 ml in diluent. Tracer (50 µl) solution was added and allowed to equilibrate for 10 min. Diluted antiserum (50 µl) was added and the tubes were incubated at 4°C for 2 hr. Diluted normal rabbit serum (50 µl) was added to the PGF assay tubes only and left to equilibrate for 10 min. Diluted DARS (50 µl) was added and the tubes incubated for 16 hr at 4°C. After the incubation, the tubes were centrifuged at 4°C, 1200 g for 45 min and 0.75 ml of the supernatant was mixed with 13 ml of scintillant and counted.

Quadruplicate zero standards, solvent blanks, non-specific blanks and counting standard tubes were used, containing 0.75 ml diluent. 50 µl of tracer was added to each tube and after 10 min equilibration, diluted anti-serum was added to each tube, with the exception of the blanks and counting standards. All the tubes were then treated like the unknown tubes, with the exception of the counting standards.

C. Cross Reaction of the Antibodies

Cross reaction of the antisera was calculated from the amount of prostaglandin required to produce a 50% fall in binding obtained with the zero standard. For example the percentage cross reaction of PGA_2 with the PGE_2 antiserum is:-

$$\frac{\text{Amount of } PGE_2 \text{ producing 50\% of zero standard binding}}{\text{Amount of } PGA_2 \text{ producing 50\% of zero standard binding}} \times 100$$

For the PGE_2 antibody, the antiserum from rabbit 7 (3rd bleed, 21 weeks after the first boost) was used at a final dilution of 1/2,000 and gave the following cross reactions:-

PGE_1	100%
PGA and B groups	2%
13,14-dihydro-15-keto PGE_2	5%

Sensitivity, defined as the amount of prostglandin required to produce a 10% fall in zero standard binding, was 30 pg/tube.

The $PGF_{2\alpha}$ antiserum was from rabbit 4 (5th bleed, 35 weeks after the first boost) and was used at a final dilution of 1/55,000 and gave

the following cross reactions:-

$PGF_{1\alpha}$	100%
$PGF_{2\beta}$	2.6%
PGD_2	3%
PGF metabolites	<1%
other PG groups	<1%

The level of detection was 45 pg/tube.

The use of silicic acid column chromatography further reduced the interference of the other prostaglandins. Since the PGE_2 and $PGF_{2\alpha}$ antibodies can not distinguish between PGE_2 and PGE_1 and $PGF_{2\alpha}$ and $PGF_{1\alpha}$ respectively, the reported concentrations are expressed as PGE and PGF.

The PGA_2 antiserum from sheep number 2 (7th bleed) was used at a final dilution of 1/2,000 and gave the following cross reactions:-

PGA_2	100%
PGA_1	21%
PGB_1	14%
PGB_2	100%
PGE_1 and 2	3%
$PGF_{2\alpha}$	<1%

The PGB_2 antiserum from rabbit number 7 (first bleed) was used at a final dilution of 1/15,000 and gave cross reactions of:-

PGB_2	100%
PGB_1	20%
$PGA_{1 \text{ and } 2}$	<5%
$PGE_{1 \text{ and } 2}$	<6%
PGF	<1%

Figures 3 and 4 show the standard curves obtained in 12 consecutive assays (mean $\pm$ s.d.) over a 6 month period.

The intra-assay precision varied between 10 and 15%.

A more detailed account of the method of raising the $PGF_{2\alpha}$ antibodies and of the radioimmunoassay method employed has been published (Dighe, Emslie, Henderson, Rutherford and Simon, 1975).

The PGA and PGB analyses were performed late in the completion of the thesis. In addition the majority of samples could not be assayed due to loss of the antibodies in a laboratory fire. Consequently the results of four animals will be briefly presented.

PG activities are presented as plasma concentrations, and are corrected for extraction recoveries.

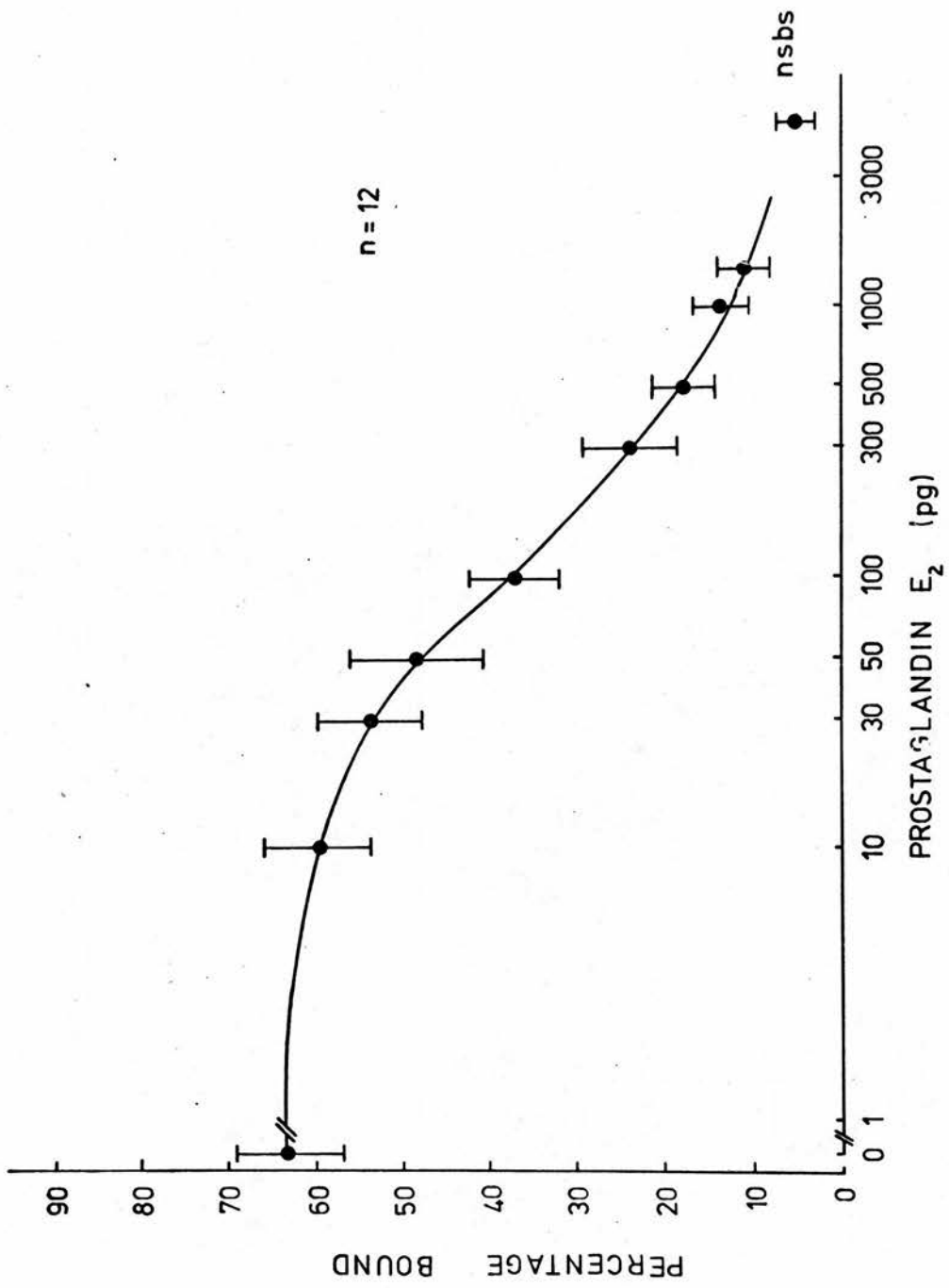

Figure 3: Standard curve for PGE_2

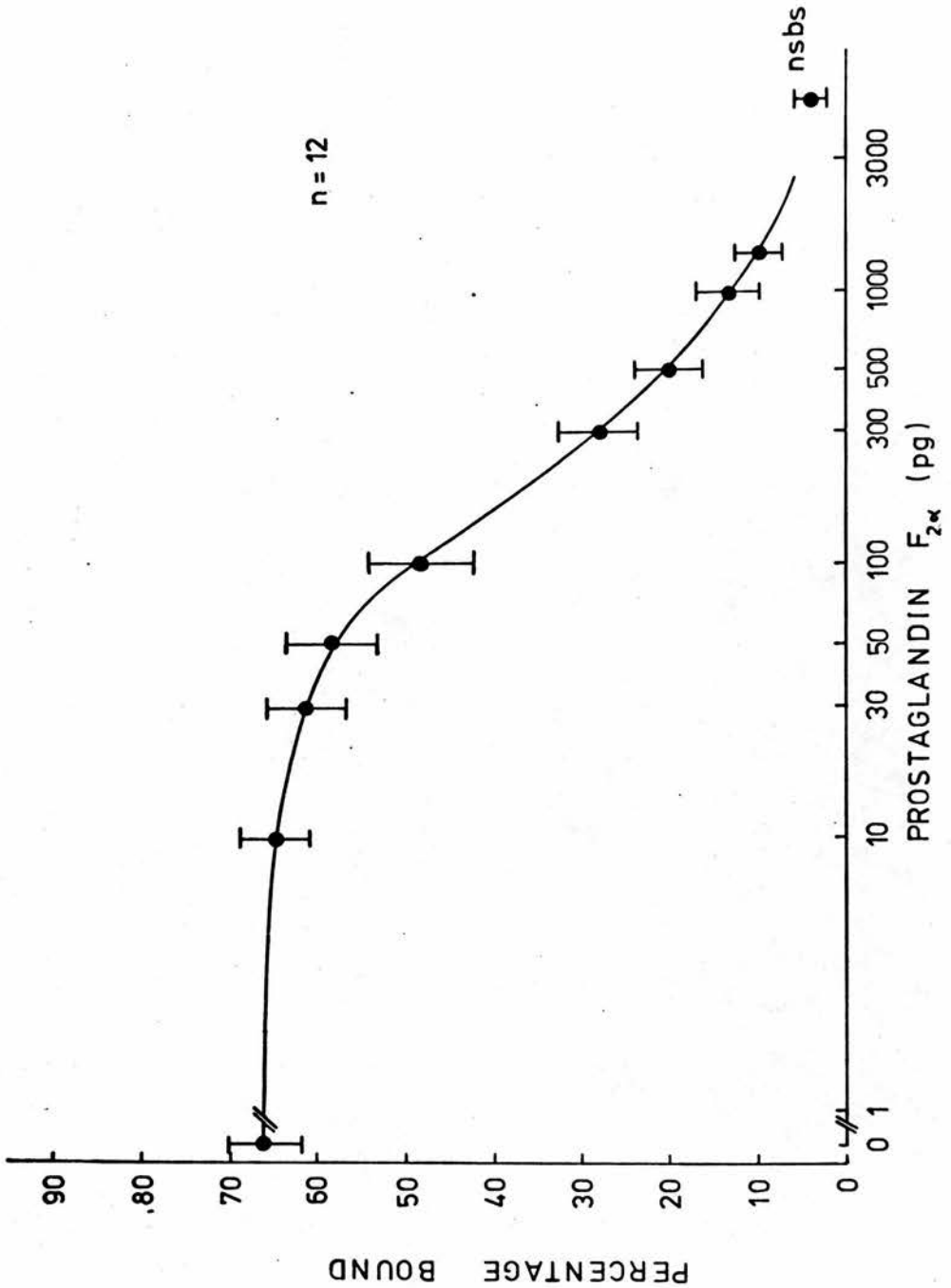

Figure 4: Standard curve for $PGF_{2\alpha}$

Renin Estimation

Plasma renin activity (PRA) was measured by means of an angiotensin I radioimmunoassay kit (CEA:IRE:SORIN, Italy), which measures the amount of angiotensin I generated during plasma incubation.

A. Blood Collection

Arterial and renal venous blood samples (10 ml) were collected in ice-cold centrifuge tubes containing 4 mg of disodium EDTA to inhibit clotting and angiotensinase. Samples were collected before, and 2, 5 and in some cases 14 and 21 days after, clamping. Each sample was centrifuged at 15,000 g for 25 min at 4°C and the plasma stored at -10°C until assay.

B. Renin Activity Estimation

Angiotensinase inhibitors (2-3,dimercaptopropanol and 8-hydroxy quinoline, 0.4 ml) were added to 0.4 ml of each plasma sample and then divided into 2 portions, one of which was incubated at 37°C and the other at 2-4°C for 1.5 hour. The latter allowed measurement of endogenous angiotensin I such that a correction could be made in estimating PRA. An optimum pH of 5.6 - 5.8 was used during the incubation.

Standard tubes of 0.5 - 8.0 ng/ml ileu-5-angiotensin I were made up in phosphate buffer using 100 µl of solution. 100 µl of ^{125}I-angiotensin I was added to each tube after 100 µl of rabbit angiotensin I antiserum, with the exception of the "total counts" tube, which

received no antiserum. The amount of antiserum used was calculated to give a binding of between 30-60% of the labelled angiotensin in the absence of unlabelled angiotensin. Incubated plasma samples (100 µl) were added to the unknown tubes. After vortexing, the tubes were incubated for 4-24 hours at 2-4°C. At the end of this period, the standard tubes were given 100 µl of "lyophilized serum" and the other tubes received 100 µl of buffer.

Dextran coated charcoal (0.5 ml) was added to each tube whilst vortexing and after 10 min the tubes were centrifuged at 1,500-2,000 g for 10 min. The supernatants (0.8 ml) were counted and from the standard curve, the amount of angiotensin I was calculated.

PRA is expressed as the amount of angiotensin I generated over a 1 hr period by 1 ml of plasma after correction for the basal angiotensin I.

It is realised that this method may not give an accurate estimation of PRA because it is assumed that the reaction kinetics are zero order even after 1.5 hours. Since no exogenous substrate was added, this may not have been true in every case.

RESULTS

Of the 12 dogs successfully taken to the clamping stage, 7 (Melody, Jolly, Flora, Apricot, Sadie, Mandoline and Whisper) became hypertensive in the days following, 1 became transiently hypertensive (Trinket) and 4 (Acorn, Jigsaw, Joyless and Topaz) failed to become hypertensive.

Post mortem examination revealed that in Joyless, the clamp had not been screwed down and consequently this animal can be considered to be sham-operated. Topaz failed to become hypertensive because the clamp was on a branch of a bifurcating renal artery. No obvious reasons for failure were evident in Jigsaw or Acorn.

Hypertensive Dogs

Grouped data is shown in Figure 5 and Table 3. Individual data is shown in Figures 6-13 and Tables A-L.

A. Blood Pressure (BP)

For the purpose of grouping the hypertensive dogs, Flora was omitted because of the anomalous behaviour of her body fluids. In the remaining 6 dogs, BP rose from 122 $\pm$ 4.4 mmHg (n=6), to 136 $\pm$ 6.3 mmHg (n=4) on the first day and reached a peak of 148 $\pm$ 5.2 mmHg (n=6), $P<0.005$, by the second day. Hypertension remained stable over the remaining days at about 142 mmHg. Thus as a group, the hypertension was sustained at about 20 mmHg above control.

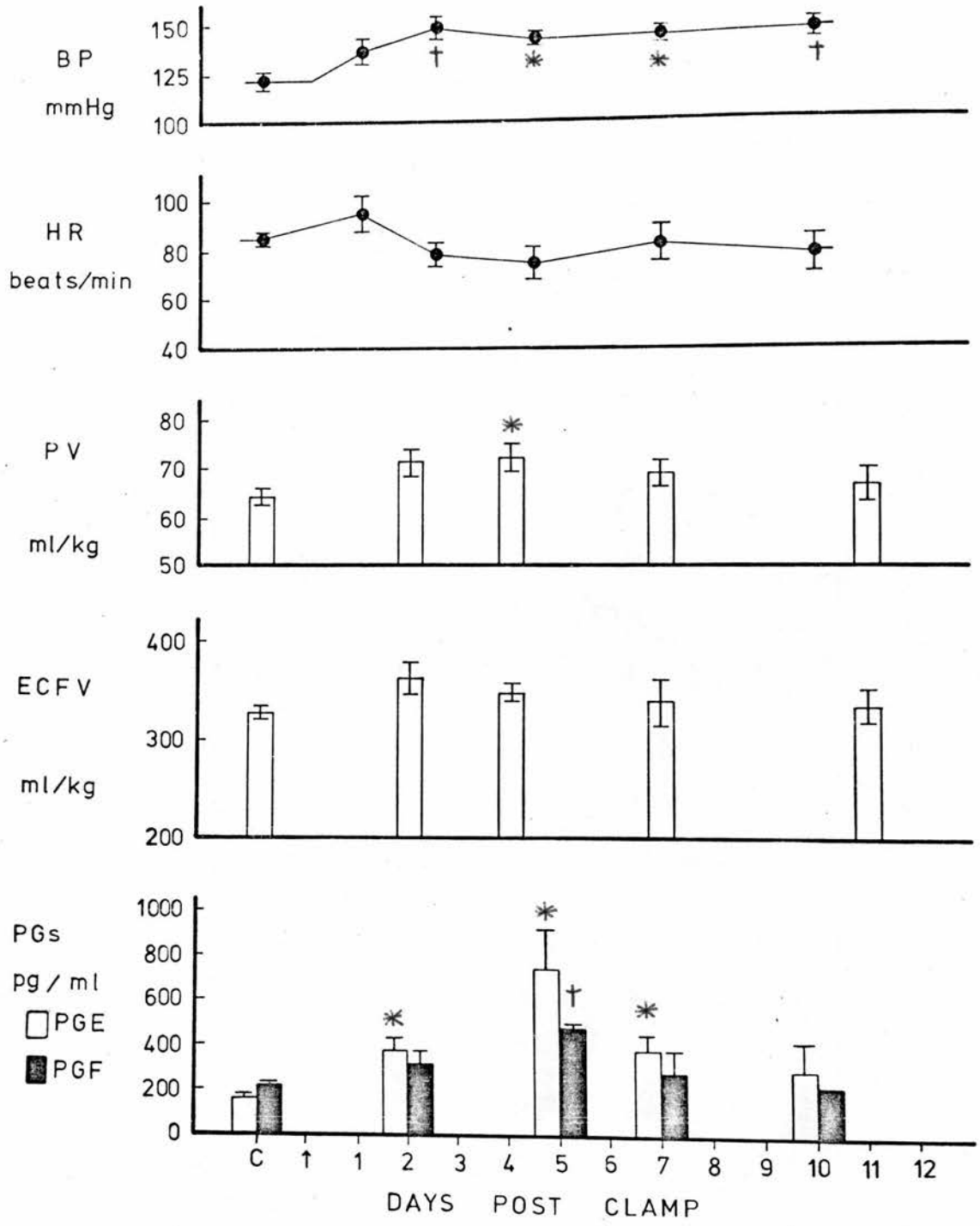

Figure 5: Grouped data from 6 hypertensive dogs. The prostaglandin data is from 3 of these dogs.
* $P<0.05$; † $P<0.01$

Table 3: Hypertensive Dogs

GROUPED DATA N is the number of paired observations

BP (mmHg)		N	P	HR (bpm)		N	P
C	123 ± 5	6		C	85 ± 3	6	
1	137 ± 7	4	ns	1	95 ± 11	4	ns
2-3	148 ± 6	6	<0.005	2-3	79 ± 5	6	ns
4-5	142 ± 4	6	<0.025	4-5	75 ± 7	6	ns
7	142 ± 6	5	<0.05	7	82 ± 8	5	ns
9-11	145 ± 6	6	<0.01	9-11	78 ± 8	6	ns
CVP (mmHg)				PV (ml/kg)			
C	3.3 ± 0.5	6		C	64.9 ± 1.8	6	
1	4.4 ± 1.2	3	<0.05	2	71.1 ± 3.2	5	ns
2-3	5.7 ± 1.4	5	ns	4	72.0 ± 2.9	6	<0.05
4-5	6.3 ± 1.7	6	<0.05	7	69.1 ± 2.9	4	ns
7	5.9 ± 1.1	4	<0.05	11	66.5 ± 3.8	5	ns
9-11	5.4 ± 0.5	4	ns				
ECFV (ml/kg)				PV/IFV			
C	327 ± 8	6		C	0.25 ± 0.01	6	
2	362 ± 19	5	ns	2	0.25 ± 0.02	5	ns
4	349 ± 10	6	ns	4	0.26 ± 0.02	6	ns
7	338 ± 28	4	ns	7	0.26 ± 0.01	4	ns
11	335 ± 19	5	ns	11	0.24 ± 0.02	5	ns
Na+ (mEq/l)				K+ (mEq/l)			
C	150 ± 3	6		C	3.9 ± 0.2	6	
2	152 ± 4	5	ns	2	3.7 ± 0.2	5	ns
4	147 ± 1	6	ns	4	3.8 ± 0.2	6	ns
7	138 ± 8	4	ns	7	3.7 ± 0.2	4	ns
11	153 ± 7	5	ns	11	4.0 ± 0.2	5	ns
PGE (pg/ml)				PGF (pg/ml)			
C	155 ± 27	3		C	209 ± 22	3	
2	364 ± 75	3	<0.05	2	315 ± 65	3	ns
5	670 ± 196	3	<0.05	5	477 ± 20	3	<0.005
7	378 ± 89	3	<0.05	7	278 ± 125	3	ns
10	290 ± 160	3	ns	10	223 ± 103	2	ns

C - control data before clamping. Figures are days after clamping.

Values mean ± S.E.

The same trend was seen in the individual dogs, although the absolute rises varied from 6 mmHg in Whisper, 9 mmHg in Mandoline, 12 mmHg in Sadie, 20 mmHg in Apricot, 34 mmHg in Melody and 38 mmHg in Jolly. All the dogs had a resting BP of between 110 and 120 mmHg, with the exception of Mandoline (145 mmHg). Flora, not included in the grouped data, had the severest hypertension of about 50 mmHg 4 days after clamping.

B. Heart Rate (HR)

Grouped HR showed no change, 85 $\pm$ 2.5 beats/min (n=6) before and 95 $\pm$ 7.2 beats/min (n=4) one day after clamping. Only 14 days after clamping did HR fall significantly below control to 74 $\pm$ 3.8 beats/min (n=3, $P<0.05$), but this may be due to the low sample number

C. Central Venous Pressure (CVP)

CVP rose from 3.3 $\pm$ 0.4 mmHg (n=6) to 5.9 $\pm$ 1.0 mmHg (n=4, $P<0.05$) 7 days after clamping but by day 16 had returned to control (4.0 $\pm$ 1.3 mmHg, n=3).

D. Fluid Volumes

All the hypertensive dogs, with the exception of Flora, showed a transient retention of fluid.

Plasma volume (PV) rose from 64.9 $\pm$ 1.6 ml/kg (n=6) to 72.0 $\pm$ 2.7 ml/kg (n=6, $P<0.05$) 4 days after clamping and returned to control over the subsequent days.

Extracellular fluid volume (ECFV) showed a similar pattern although the peak increase was noted earlier, 2 days after clamping, having risen from 327 ± 7.5 ml/kg (n=6) to 362 ± 17.2 ml/kg (n=5) and 348 ± 9.1 ml/kg (n=6) 2 and 4 days after clamping respectively. In both cases the rises just failed to reach the 5% significance level ($P<0.1$ in both cases).

No change in the distribution of extracellular fluid as judged by PV/IFV could be detected, with a control of 0.24 ± 0.11 (n=6) and 0.26 ± 0.01 (n=6), 4 days after clamping.

D. Plasma Electrolytes

Plasma sodium and potassium concentrations did not change significantly from control values of 150 ± 2.6 mEq/litre (n=6) and 3.89 ± 0.18 mEq/litre (n=6) respectively.

E. Renal Venous Prostaglandins

In 3 dogs, which became hypertensive and in which the renal venous catheters were still functional (Jolly, Melody and Mandoline), a transient rise in renal venous PGE and PGF concentrations occurred. PGE concentrations rose from a control of 155 ± 27 pg/ml (n=3) to 364 ± 75, 670 ± 196 and then fell to 378 ± 89 pg/ml (all n=3 and $P<0.05$), 2, 5 and 7 days respectively after clamping. By the tenth day PGE levels had returned to control, 291 ± 160 pg/ml (n=3).

PGF rose also from 209 ± 22 pg/ml to 315 ± 65, 477 ± 20 pg/ml and then fell to 278 ± 125 pg/ml (n=3) 2, 5 and 6 days after clamping and had returned to 223 ± 103 pg/ml (n=2) 10 days after clamping. Only the rise on day 5 reached significant levels ($P<0.005$).

In each of these 3 dogs, control PGF concentrations exceeded the PGE concentrations, but with the onset of hypertension the PGE concentrations exceeded the PGF concentrations, with the exception of Jolly.

Both Jolly and Melody showed a sharp rise in both prostaglandins, reaching a peak 5 days after clamping. PGE rose to 2.3 and 7.5 fold of control from controls of 130 and 129 pg/ml and PGF rose to 2.6 and 2.5 fold of control from 200 and 175 pg/ml respectively. See Figures 6 and 7.

PGE rose biphasically in Mandoline, peaking at days 5 and 10, but this may be due to a false low estimate on day 7 or a false high estimate on day 10. The peak PGE on day 5 was 735 pg/ml, 3.5 fold that of control (210 pg/ml) and PGF rose to a peak of 525 pg/ml, 2.1 fold that of control (250 pg/ml). See Figure 8.

In Sadie, in which the clamped kidney's renal venous blood was sampled, PGE and PGF levels rose, but peaked earlier, at 1 and 2 days after clamping, rising to 535 pg/ml and 280 pg/ml, 2.35 and 2.61 fold of control for PGE and PGF respectively. By the 5th day PGE levels returned to control, whereas PGF remained elevated (Figure 9).

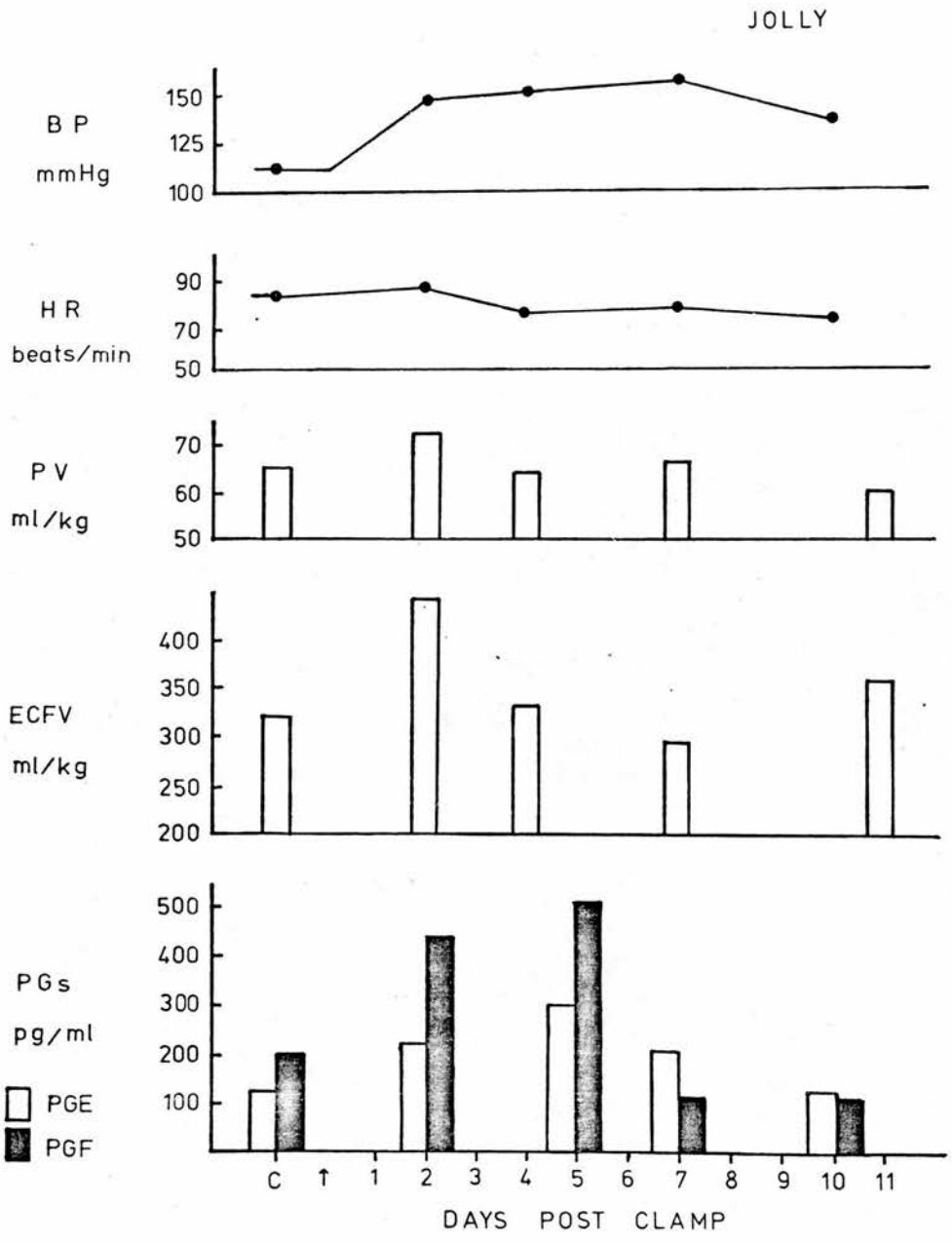

Figure 6: "Jolly"

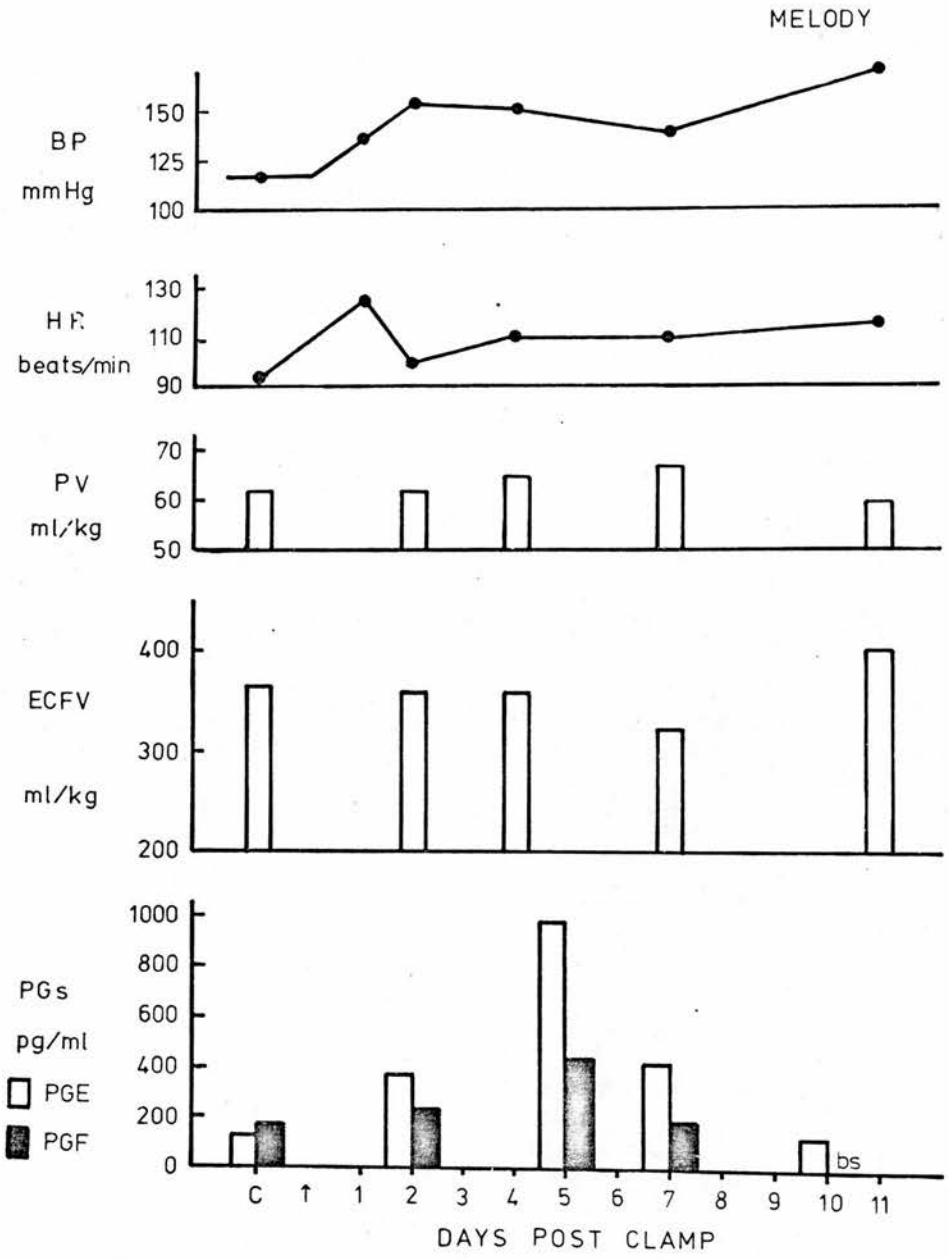

Figure 7: "Melody"

bs = below the sensitivity of detection

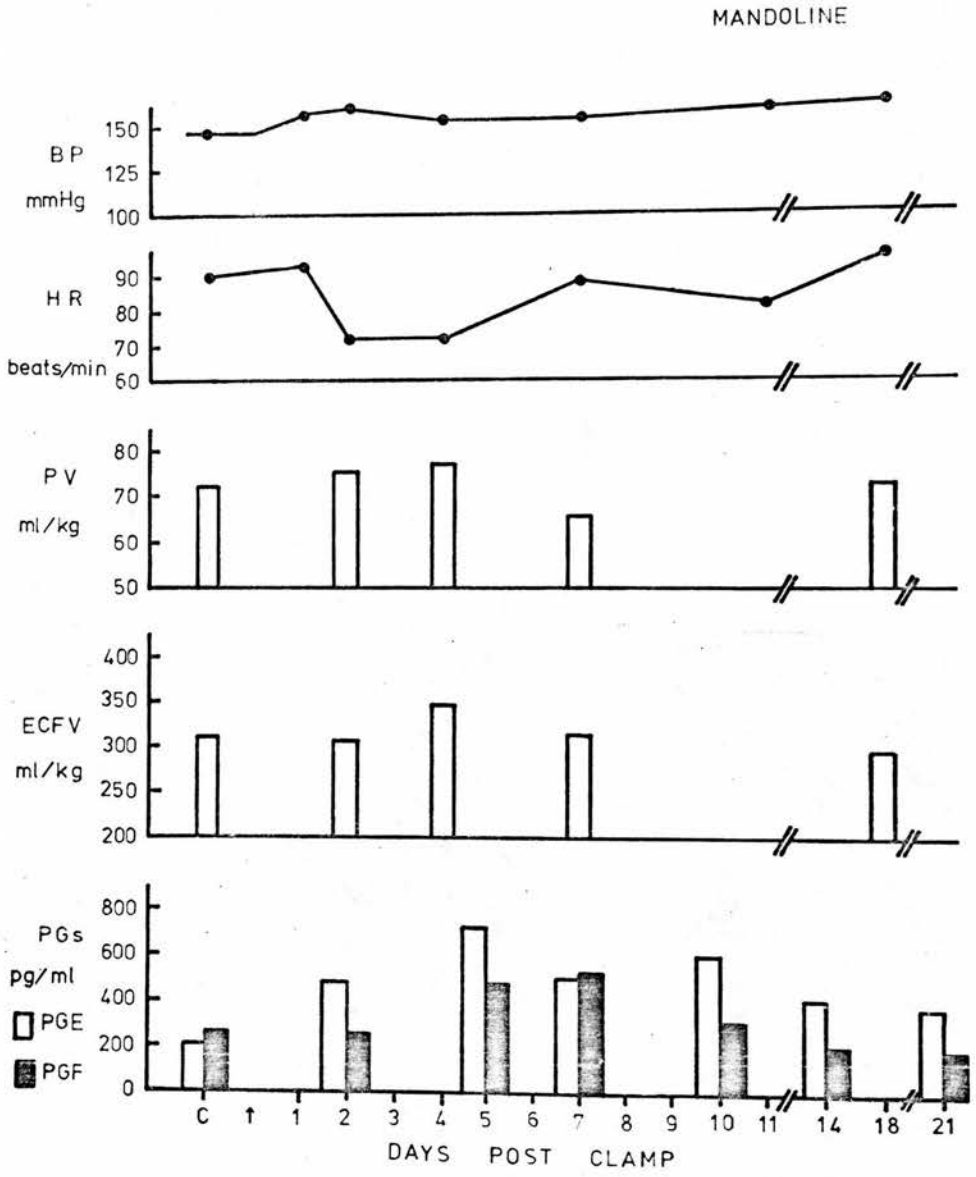

Figure 8: "Mandoline"

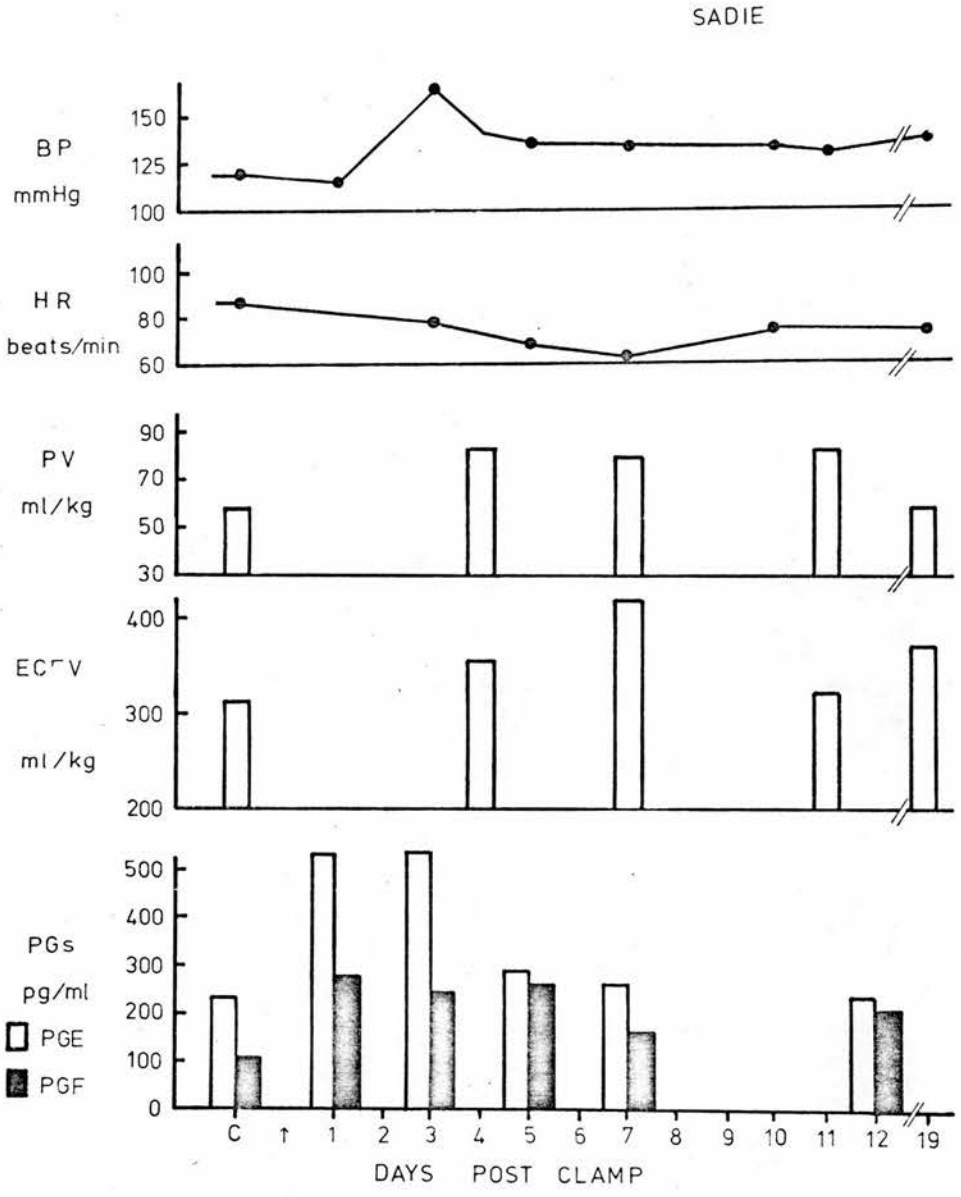

Figure 9: "Sadie" In this dog the renal venous blood from the kidney opposite the clamped kidney, was sampled.

PGA and PGB levels were measured in Jolly and Sadie. Unlike the renal venous PGE and F concentrations, PGA levels in fact fell with the progression of hypertension in Jolly, from 600 to 330 pg/ml and below sensitivity, whereas PGB rose from 420 pg/ml reaching a peak of 1035 pg/ml at day 7 (Table A).

The renal venous plasma from the ipsilateral kidney of Sadie showed a biphasic rise in PGA and PGB, rising from 1415 and 2730 pg/ml respectively to 2820 and 2790 pg/ml, 3 and 7 days post clamp for PGA and 4420 and 4510 pg/ml on days 3 and 12 for PGB, unlike PGE and F, which peaked on day 1 only (Table D).

Arterial PGE and F levels of these dogs failed to show a trend, unlike the PGA and B levels, which closely followed the renal venous levels in time course and levels.

F. Renin Activities

A rise in the arterial renin activities was seen in Melody and Mandoline rising from 2.1 to 2.8 and 3.6 to 8.0 ng ml^{-1} hr^{-1} of angiotensin I respectively 2 days after clamping. The levels quickly returned to normal and by the 5th day were indistinguishable from control (1.1 and 2.0 ng ml^{-1} hr^{-1} angiotensin I respectively). With the onset of hypertension renin release was suppressed in the untouched kidney as judged by the VA differences (Table 4).

Flora (Figure 10) showed the severest hypertension, with a rise in BP from a mean control of 128 mmHg to 170 mmHg 5 days after clamping. Thereafter BP varied between 133 and 152 mmHg between days 7 to 17. HR showed no consistent changes.

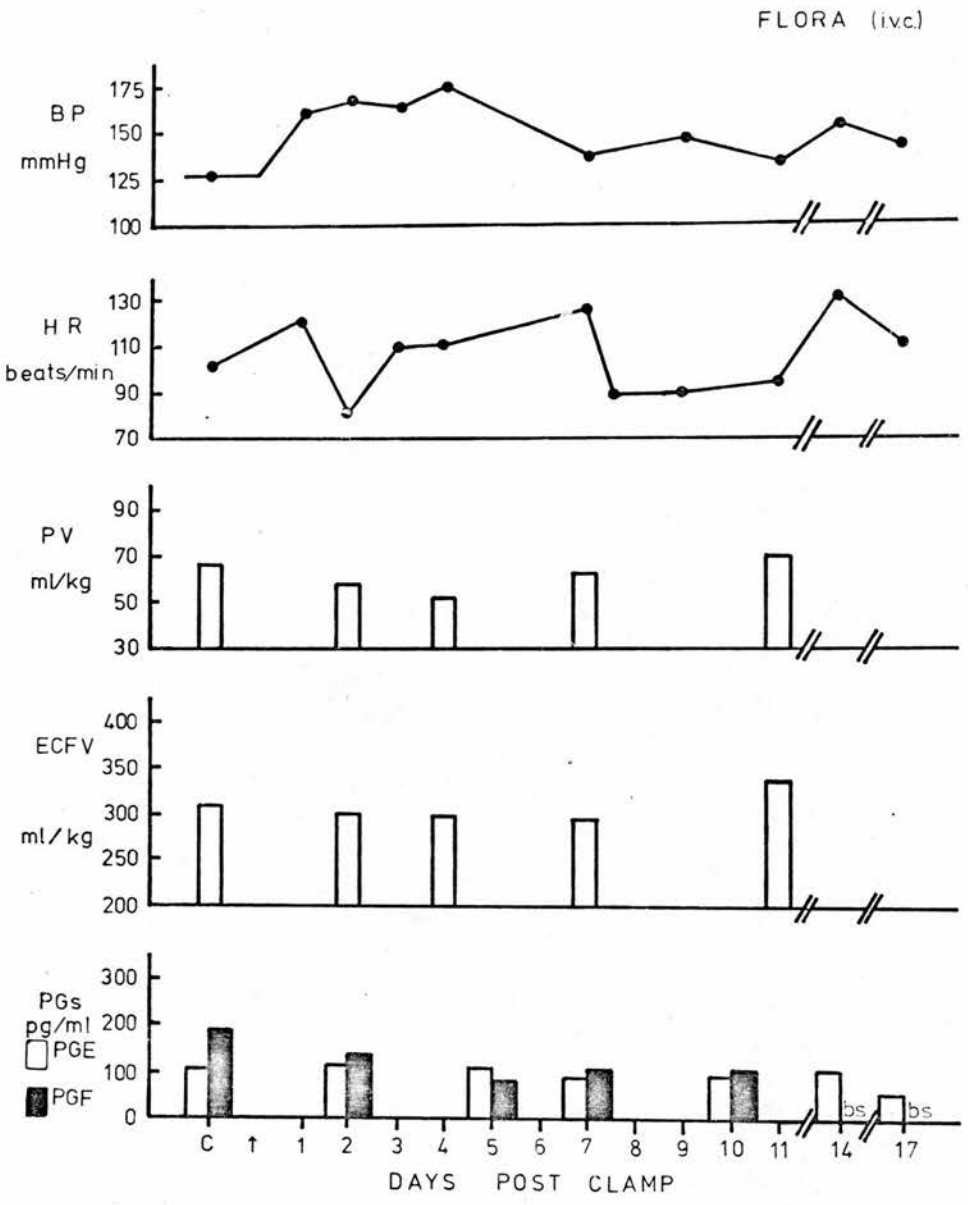

Figure 10: "Flora" Blood from the inferior vena cava was sampled for prostaglandin activity

Table 4: Arterial Renin Activity (ARA)

Dog	Day	ARA	VA difference
Melody	C	2.1	-0.3
	2	2.8	-0.3
	5	1.1	+1.4
Mandoline	C	3.6	+0.4
	2	8.0	-3.2
	5	2.0	+1.2
	14	2.4	0
	21	4.8	-1.2
Apricot	C	2.8	-
	2	12.4	-
	5	10.0	-
Acorn	C	1.2	+0.4
	2	6.0	0
	5	2.8	-0.8
	14	1.2	+0.8

Dogs Melody, Mandoline and Apricot became hypertensive, however no renal venous blood was sampled in Apricot. Acorn did not become hypertensive but she lost 50% of her blood volume on day 1.
ARA is expressed in ng ml^{-1} hr^{-1} of generated angiotensin I.
The VA difference is the renal venous concentration (contralateral kidney) minus the arterial concentration and is an index of renin secretion.

Both PV and ECFV fell with the onset of hypertension, unlike the other hypertensive dogs. After days 5-7 fluid retention occurred.

PGE and F renal venous concentrations did not deviate from control levels of 110 and 190 pg/ml respectively. However, at post mortem, the catheter tip was found to be lying in the inferiour vena cava as suggested by the ivp taken 3 days after clamping.

PGA and B concentrations rose transiently in the "renal venous" and arterial plasma of this dog reaching a peak on the 5th day. PGA showed almost a 10-fold rise in "renal venous" levels from 1500 pg/ml to 10,400 pg/ml, whereas the rise in PGB was smaller, 565 pg/ml to 2,705 pg/ml. Arterial levels of both these prostaglandins showed a similar time course of changes (Table E).

Similarly in Apricot (Figure 11) the renal venous catheter was found to be sampling vena caval blood. Despite hypertension, inferior vena caval plasma PGE and PGF concentrations did not change from control (128 pg/ml and 140 pg/ml respectively). PGA and PGB arterial and vena caval concentrations transiently rose, peaking on day 2. The vena caval PGA levels rose from 680 pg/ml to 1,050 pg/ml and PGB rose from 145 pg/ml to 710 pg/ml (Table F).

Arterial renin activity rose transiently, peaking at day 2, having risen from a control of 2.8 $ng.ml^{-1}hr^{-1}$ angiotensin I to 12.4 $ng.ml^{-1}hr^{-1}$ angiotensin I (Table 4).

Due to a fault in the storage freezer the plasma from the hypertensive dog, Whisper and the non-hypertensive dog, Topaz, failed

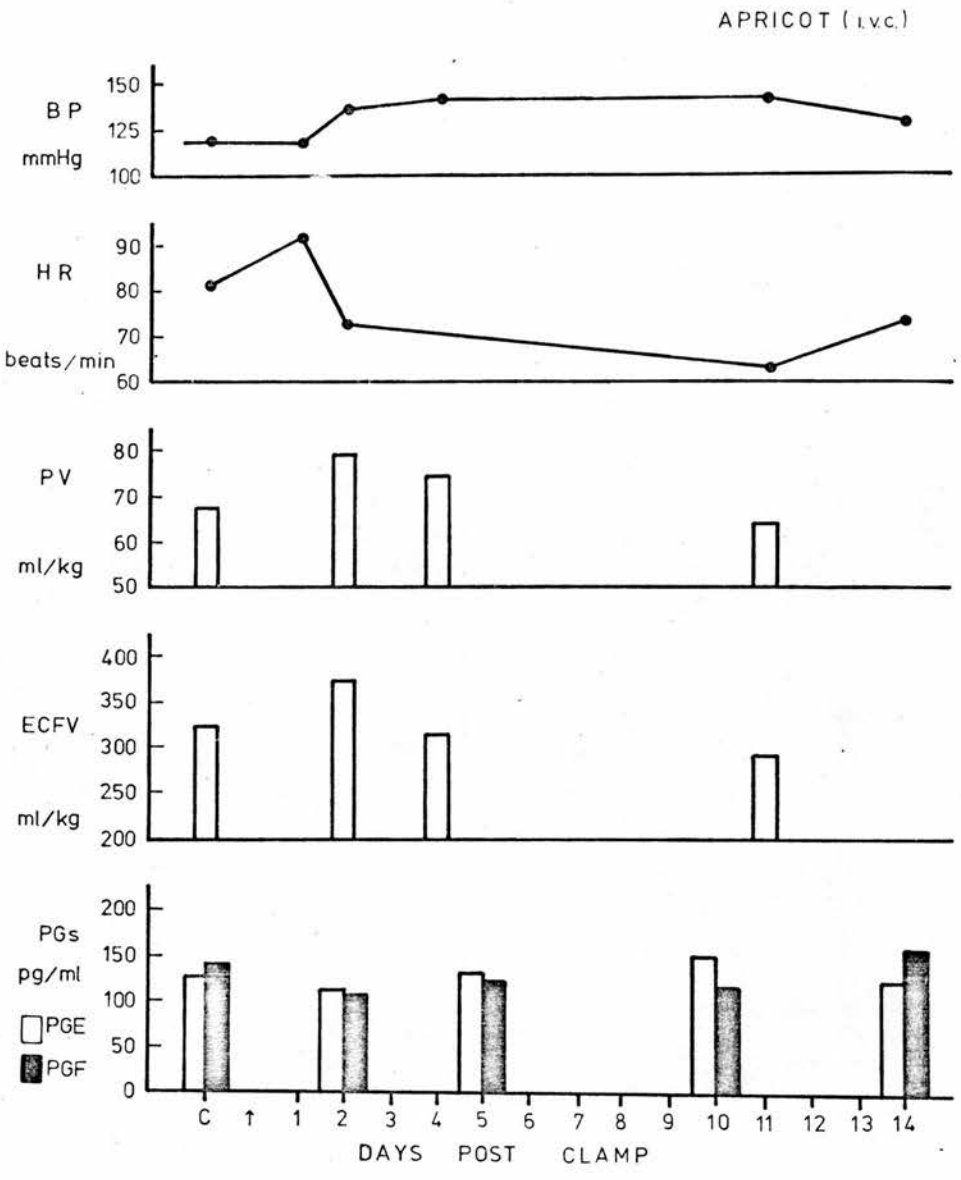

Figure 11: "Apricot" Blood was sampled from the inferior vena cava for prostaglandin activity.

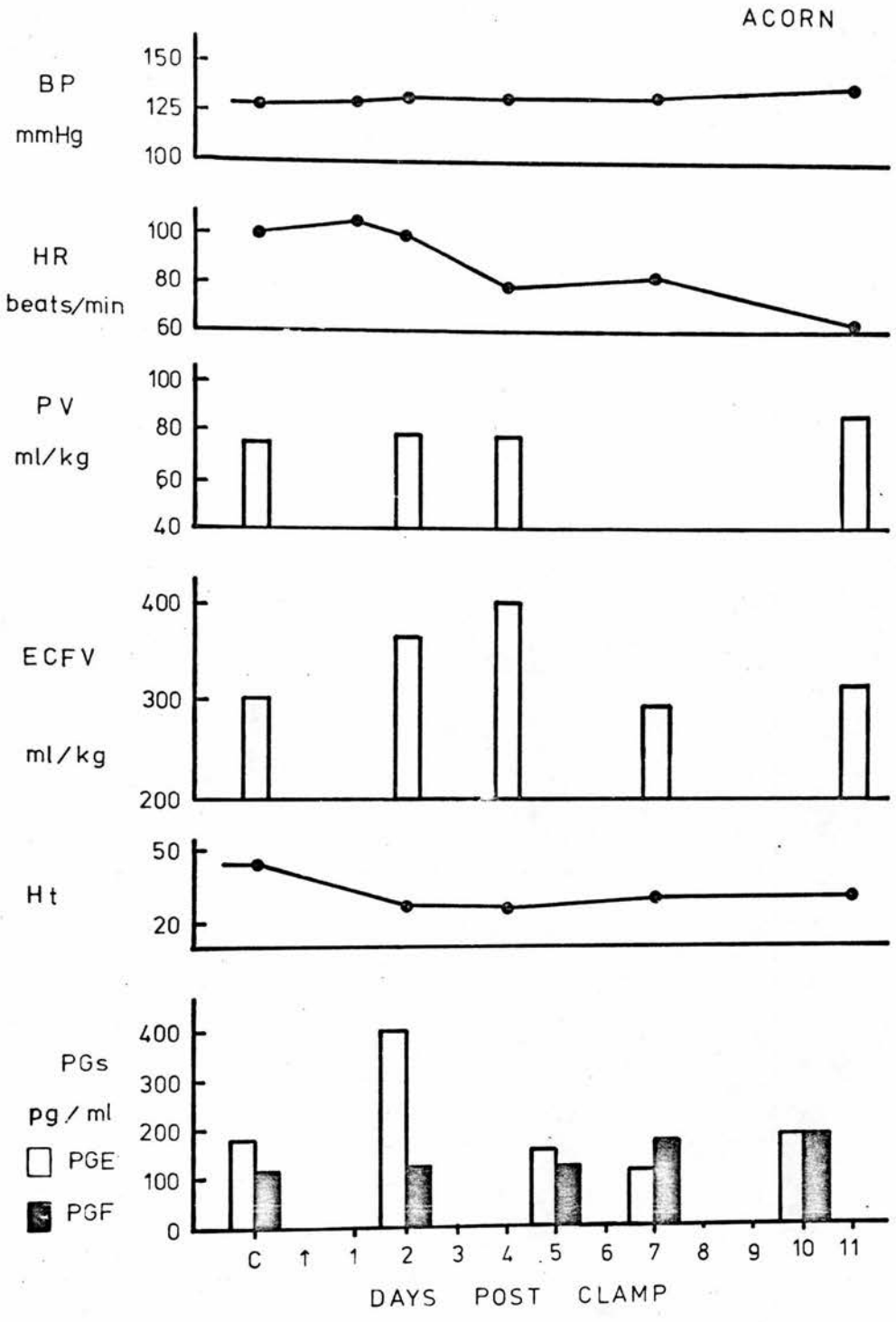

Figure 12: "Acorn" This dog failed to become hypertensive but on the day following clamping lost 50% of her blood volume.

freeze properly resulting in a loss of prostaglandin activity. The ria and extraction procedure were absolved from blame since 4 samples from Trinket, repeated because of poor replication, gave reasonable results.

Non-hypertensive Dogs

In one dog, Acorn, (Figure 12) the arterial catheter obturator accidentally came loose, the day after clamping, resulting in a 50% loss of blood volume.

Red cell volume, calculated from PV and Ht, fell from 55.4 ml/kg to 28.9 ml/kg. PV rose from 72.1 to 78.4, 77.5 and 85.7 ml/kg, 1, 2 and 10 days later, whilst ECFV rose transiently from 300 to 367 and 401 ml/kg, 1 and 2 days after and then fell to control, 314 and 308 ml/kg, 10 and 17 days after the "haemorrhage" as BV and Ht returned to normal.

Renal Venous Prostaglandin Concentrations

Renal venous prostaglandins showed no change with the exception of a large transient increase in PGE only from 180 pg/ml to 400 pg/ml 1 day after the "haemorrhage", well outside the intra-assay covariance limits (10-15%). Arterial prostaglandin concentrations showed no trend.

Renin Activities

The day after the "haemorrhage" (day 2) as the renal venous PGE reached a transient peak, arterial PRA also showed a sharp rise

from 1.2 ng $ml^{-1}hr^{-1}$ angiotensin I to 6.0 ng $ml^{-1}hr^{-1}$ angiotensin I, falling to 2.8 and 1.2 ng $ml^{-1}hr^{-1}$ angiotensin I on days 5 and 14 respectively (Table 4).

A transient rise in BP was seen in Trinket (Figure 13), with BP rising from 110 to 126 mmHg, 2 days after clamping, whilst PV and ECFV transiently rose from 63.4 to 69.2, and 337 to 365 ml/kg, 2 days after clamping respectively. CVP rose from 4.4 to 6.7 mmHg and HR fell from 89 to 56 beats/min and thereafter all parameters returned to normal. Renal venous PGE and PGF concentrations rose from a mean of 185 and 106 pg/ml to 352 and 360 pg/ml respectively 2 days post clamp and did not return to control even by the 10th day (258 and 296 pg/ml respectively).

No hypertension was seen in Jigsaw and as the carotid catheter had entered the left ventricle, the experiment was terminated (Table L).

Topaz also failed to become hypertensive (BP of 106 mmHg) and PV and ECFV rose from mean controls of 71.6 and 336 ml/kg to 75.2 and 357 ml/kg 4 days after clamping. HR did not change and CVP fell to 50% of control from 4.3 mmHg before rising again to control. Due to the freezer fault no prostaglandin levels were detectable (Table J).

Generally there was about a 2:1 ratio of renal venous PGE to PGF as shown in Figure 14, taken from 7 dogs, which agrees with the figures reported for the kidney (Davis and Horton, 1972; Lonigro et al 1973).

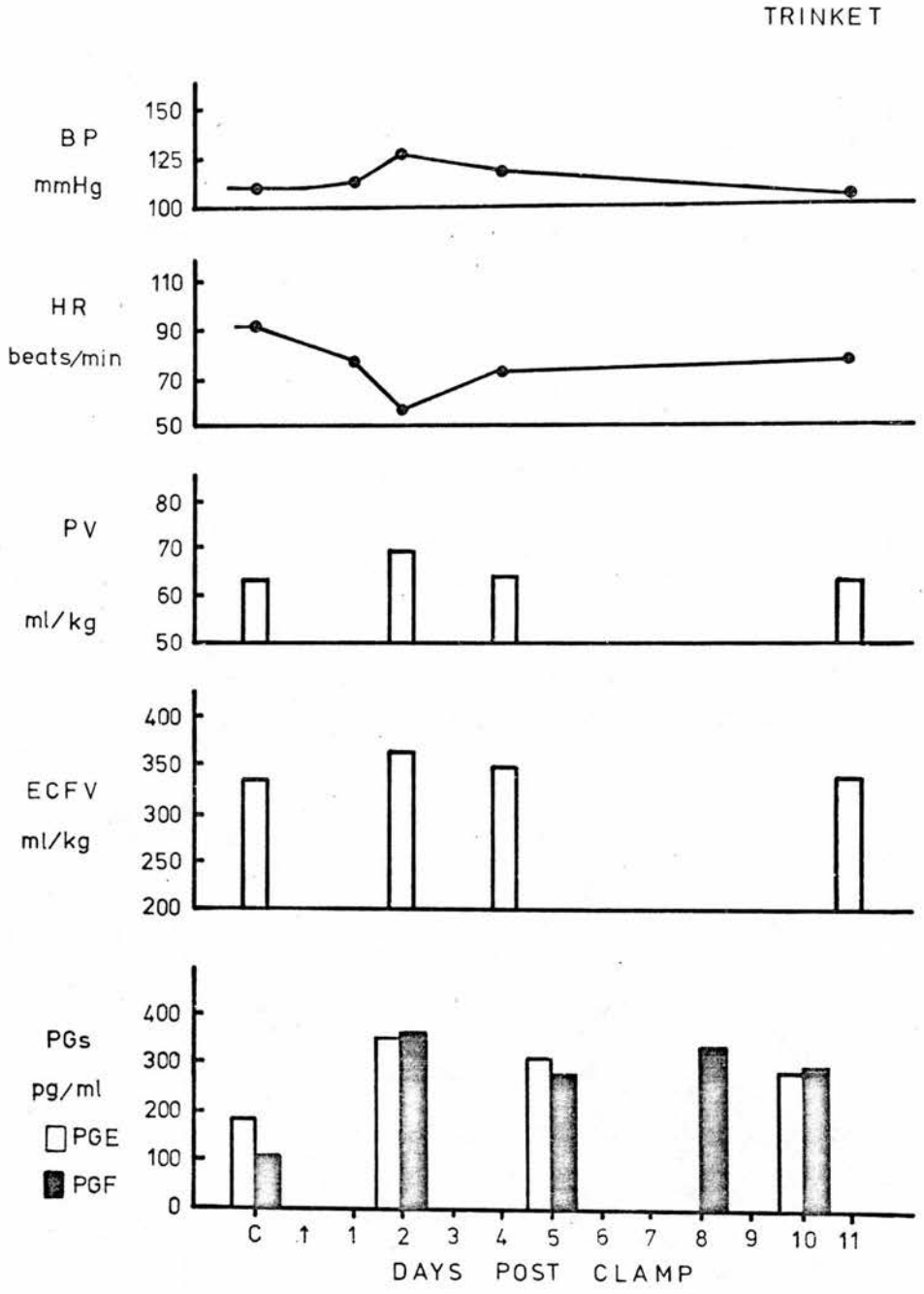

Figure 13: "Trinket" Only transient hypertension was seen in this dog.

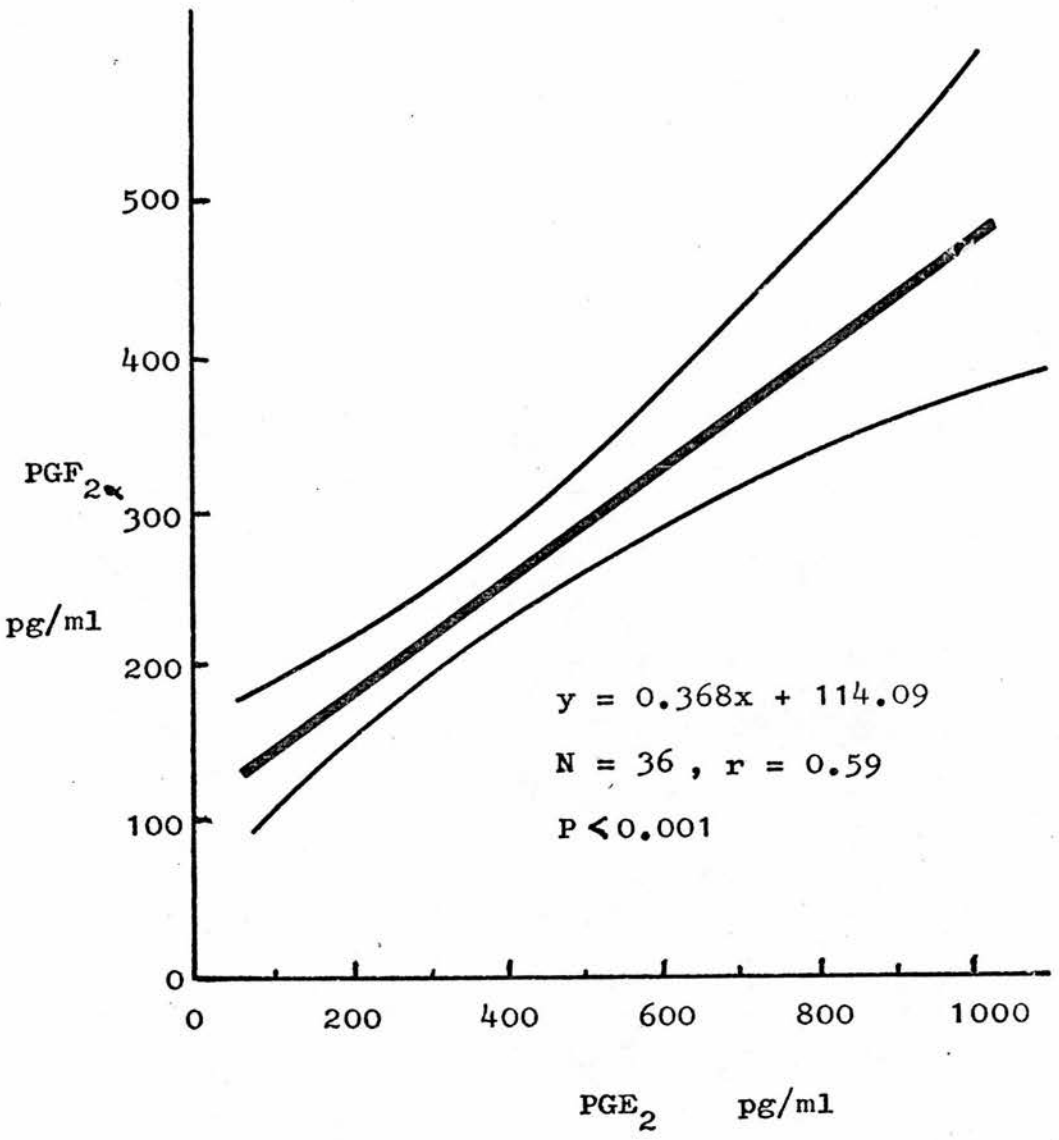

Figure 14: Correlation of renal venous PGE_2 and $PGF_{2\alpha}$ in 7 conscious dogs. The 95% confidence limits are shown.

Indomethacin Treatment

Indomethacin (Indocid, Merck, Sharpe and Dohme, 50 mg orally, bid) failed to alter the BP of a non-hypertensive dog, Joyless, when given on day 7 after clamping, for 3 days, despite a fall in central venous PGE and PGF from 515 to 210 pg/ml and 216 to 160 pg/ml respectively. Similar pretreatment in a hypertensive dog, Whisper, for 4 and 5 days also failed to alter BP (see Tables K and I respectively).

PGE_2 Infusion

PGE_2 was infused into Jigsaw and Whisper to study the effects of acute infusions at rates comparable to peak renal release. The largest change in renal release of PGE was seen in Melody, with a peak of 973 pg/ml, 5 days after clamping. Assuming a Ht of 50 and RBF of 10 ml $min^{-1}kg^{-1}$, then this is equivalent to a release of 5 ng $min^{-1}kg^{-1}$ (iv).

PGE_2 (in methanol) was diluted in saline and infused at 1 ml/min to give infusion rates of 13 and 132 ng $min^{-1}kg^{-1}$ in Jigsaw. Neither affected BP, nor did a bolus injection of 10 µg.

Infusion of 13 ng $min^{-1}kg^{-1}$ and even 1310 ng $min^{-1}kg^{-1}$ produced no effect in Whisper. Bolus injections of 24 µg did not alter BP and 48 µg and 240 µg produced falls of 38 and 74 mmHg respectively.

DISCUSSION

Following partial constriction of one of the renal arteries, the resultant mild arterial hypertension in dog is accompanied by a transient rise in PRA and fluid retention, in agreement with previous work (Bianchi et al, 1972). The immediate rise in BP has been shown to be due to the rise in circulating angiotensin II (Bianchi, Tenconi and Lucca, 1970; Bianchi et al, 1972; Miller, Samuels, Haber and Barger, 1972; Caravaggi, Bianchi, Brown, Lever, Morton, Powell-Jackson, Robertson and Semple, 1976). Within a few days and in this project, by the fifth day, the renin levels had returned to normal, despite the persistence of hypertension (Brown, Davis and Olichney, 1966; Bianchi et al, 1970, 1972; Harris and Ayers, 1972; Zimmerman, 1973; Miller, Samuels, Haber and Barger, 1975). At this time, converting enzyme inhibition and angiotensin antagonists became ineffective at lowering BP (Miller et al, 1972, 1975; Bumpus, Sen, Smeby, Sweet, Ferrario and Khosla, 1973).

The expansion of the vascular compartment, which reached a maximum by the fourth day in these experiments, has been suggested to be the cause of the elevated cardiac output seen in this model (Bianchi et al, 1972). A similar hypertinetic circulation is reported early in the development of hypertension in one-kidney Goldblatt dogs (Bianchi et al, 1970; Ferrario, 1974), cellophane perinephritic dogs (Ferrario, Page and McCubbin, 1970), one-kidney Goldblatt rats (Ledingham and Cohen, 1962, 1964; Ledingham and Pelling, 1967), the renoprival rat (Ledingham and Pelling, 1970) and in the Japanese SHR (Pfeffer and Fröhlich, 1973). It is

believed that the resultant overperfusion of the body leads to "whole body autoregulation" by elevation of peripheral resistance and that this perpetuates the hypertension as cardiac output returns to normal (see Guyton and Coleman, 1969).

Somewhat later than the rise in PRA, which reached a maximum 2 days after clamping, contralateral renal venous PGE and PGF concentrations rose, reaching a peak on the fifth day. Contralateral renal venous PGA fell in one dog (Jolly), whereas PGB rose but peaked 2 days later than the E and F levels. Vena caval and arterial concentrations of PGA and B in two other hypertensive dogs, transiently rose, peaking at day 2 in the case of Apricot and day 5 in Flora.

In the absence of renal blood flow figures it is assumed that these changes mirror increases in prostaglandin synthesis and release from the kidney. Contralateral renal blood flow does decrease acutely after clamping (McGiff et al, 1970a; Zimmerman, 1973) due to the rise in PRA (Satoh and Zimmerman, 1975b) but the fall is small and certainly considerably less than 50% reduction. Since the renal venous prostaglandin concentrations rose more than two-fold and these changes occurred as PRA reached control levels, then it is likely that the concentration changes were not due to dilution changes caused by flow reduction, but are due to genuine changes in synthesis.

Angiotensin II has been shown to release prostaglandins from the kidney (McGiff et al 1970; Aiken and Vane, 1973; Needleman et al, 1973; Danon et al, 1975) but as this is an immediate effect and in the present experiments the plasma changes in angiotensin

precede those of prostaglandins by about three days, then this is unlikely to be the mechanism of the prostaglandin release.

It is more likely that prostaglandin synthesis in the untouched kidney is stimulated by the expansion of the vascular compartment, secondary to the elevated PRA. There is a vast amount of evidence implicating renal prostaglandins in fluid and salt excretion control. Prostaglandins of the E and A series and arachidonic acid produce natriuresis and diuresis when infused into the canine kidney (Fülgraff et al, 1974; Tannenbaum et al, 1975) and indomethacin depresses sodium and urine excretion (Feigen et al, 1976; Olsen et al, 1976). Injection of sodium chloride into the renal artery of dog has been reported to release PGE (Jubiz et al, 1976) but there are as yet no reports on the effects of volume expansion in this species.

Prostaglandin infusions in rat also cause natriuresis, although urine osmolality falls (Fülgraff and Meiforth, 1971) and an essential fatty acid deficient diet (Rosenthal et al, 1974), aspirin (Susic and Sparks, 1975) and indomethacin (Düsing et al, 1976) all prevent the excretion of a saline load.

Blood volume expansion in rat leads to a rise in plasma prostaglandins, which is prevented by nephrectomy (Papanicolou, 1972, 1975) and urine prostaglandin excretion is also reported to rise (Papanicolaou et al, 1976).

It seems plausible that the contralateral kidney responds to the fluid retention by increasing its prostaglandin production, leading ultimately to salt and water excretion and a return of fluid and

electrolyte homeostasis.

Another alternative, which must be considered, is that the renal prostaglandins are in fact natriuretic. In the conscious dog meclofenamate and a competitive synthetase inhibitor (RO 20-5720) cause natriuresis (Kirschenbaum and Stein, 1976) and indomethacin has been reported to cause natriuresis in the isolated canine kidney (Vankerweghem et al, 1975). These two observations could explain why there is an inverse relationship between sodium intake and peripheral plasma PGA in man (Zusman et al, 1973a; Lee and Attallah, 1974), rat (Zusman et al, 1973b) and in rabbit kidney (Tobian, 1974). However these latter observations may be explained by the fact that angiotensin may be causing prostaglandin release.

It is unlikely that the contralateral kidney participated in fluid retention by increasing its prostaglandin activity since the fluid retention preceded the rise in prostaglandin synthesis.

Unfortunately due to lack of time and insufficient results, the effect of a prostaglandin synthetase inhibitor during the crucial first few days after clamping could not be investigated. In addition the prevention of the fluid retention by dialysis would allow the hypothesis, that this causes the release of renal prostaglandins to be tested. Investigation of this nature is to be undertaken in this laboratory by another researcher.

There is, however, little doubt that the rise in prostaglandin activity seen in the contralateral kidney is not a general occurrence since there was no rise in the mixed venous blood

from the inferior vena cava of Flora or Apricot, despite the presence of hypertension.

It has been demonstrated in the Goldblatt one-kidney rat that the hypertension is accompanied by a selective expansion of the vascular compartment, with a normal interstitial fluid volume (IFV) due to a fall in tissue compliance. Unclipping the renal artery led to a fall in BP and a rise in tissue compliance (Lucas and Floyer, 1974). In the conscious dog there is a preferential retention of fluid in the vascular compartment of anephric dogs after saline loading compared with dogs with uninephrectomy and ureterocaval anastomosis of the other kidney (Liard, 1976).

The possibility of a humoral mechanism of renal origin, controlling fluid distribution, has been made. This effect may partially or even wholly explain the antihypertensive action of the kidney.

The absence of a change in the PV/IFV ratio in the present study may be argued as evidence that the renal control of body fluid distribution was functioning adequately resulting in only a mild form of hypertension. If this were so, then the greatest demand on the kidney would be when fluid retention was at its peak. At this time prostaglandin output is also elevated to its maximum.

Using the data of Bianchi et al (1970; 1972), there was found to be no change in the PV/IFV ratio in the two-kidney dog, but a large prolonged increase was present in the one-kidney dog. This strongly suggests that the presence of normal, but not necessarily excreting renal tissue, does affect fluid distribution and in the direction required by the antihypertensive kidney theory.

The pulmonary barrier to PGE (and PGF) (Vane and Ferreira, 1967; Robertson, 1975) should prevent extrarenal effects, unless of course the metabolites are bio-active. The efficiency of this barrier was not reduced in these hypertensive dogs since PGE_2 infusions as high as 260 times the calculated peak release from the untouched kidney, failed to acutely alter BP. In addition indomethacin failed to alter BP in the normotensive and hypertensive dog. To date, only one successful report of chronic administration of indomethacin producing chronic hypertension has appeared (Colina-Chourio et al, 1975). However, another group has failed to demonstrate this using the same species, rabbit (Muirhead et al, 1976).

Prostaglandin of the A and C series would not be hindered by the pulmonary barrier, however. The few results presented of PGA, show a rise in circulating PGA which, in view of its stability in the pulmonary circulation, has been suggested as a circulating hormone. Consequently, it might be suggested that PGA could affect various parts of the cardiovascular system and be responsible for the distribution of the extracellular fluid.

The levels of PGA reported are in the subnanogram and nanogram per ml level. Using the data of Fülgraff et al (1974), McClatchey and Carr (1973) and Tannenbaum et al (1975), these are pharmacological levels of PGA, producing natriuresis and increasing blood flow of the kidney.

However, in view of the scepticism regarding the natural existence of PGA and the fact that the PGA antibodies main cross reaction is

with PGB_2 at 100%, then it would be more cautious to think of the reported PGA levels in terms of immunoreactive PGA.

In one case where the clamped kidney was sampled, a very rapid rise then fall of prostaglandin renal venous concentrations was recorded, within two days of clamping. In the absence of RBF data the results are difficult to interpret since RBF would be expected to fall due to clamping (Bounous and Shumacker, 1962; Lupu, Maxwell, Kaufman and White, 1972; Harris and Ayers, 1972; Ferrario and McCubbin, 1973) and this would raise renal venous concentrations.

Chronically, RBF does return to control again (Corcoran and Page, 1942; Bounous and Shumacker, 1962) but it is not known how rapidly this occurs in the two-kidney Goldblatt dog. In the one-kidney Goldblatt dog up to 7 days are required (Ferrario and McCubbin, 1973).

McGiff et al (1970) and Herbaczynska-Cedro and Vane (1973) believe that acute renal ischaemia causes prostaglandin release from that kidney. However, taking into account the fall in RBF, Beckman and Zehr (1975) found a fall in release, although in agreement with the previous two groups, they found a rise in concentrations in the renal venous blood. Moreoever, Gross, Mujovic, Jubiz and Fisher (1976a) produced a 12 hr long 30% reduction in RBF in the anaesthetised dog and found that the renal venous PGE concentration rose slowly to 200% of control by the 8th hour. This is not an increase in synthesis as the authors suggest since there was no immediate tripling of concentration, but is a depression of synthesis

with a slow recovery, reaching 66% of control by the 8th hour.

Irrespective of whether prostaglandin synthesis in the ischaemic kidney does fall, it is probable that intrarenal prostaglandin concentrations rise at certain sites and this probably attenuates the vasoconstrictor and antidiuretic activities of angiotensin in the kidney (Aiken and Vane, 1973).

According to Satoh and Zimmerman (1975a), the elevated angiotensin activity during renal ischaemia causes a rise in intrarenal prostaglandin activity and since angiotensin I (Needleman et al, 1973) and angiotensin II (McGiff et al, 1970a; Needleman et al, 1973) both release renal prostaglandins, then this may explain this observation in the ischaemic kidney.

PGA_1 has been shown to antagonise the excretory changes produced by acute renal artery stenosis in the dog (McClatchey and Carr, 1973), whilst in the chronically ischaemic canine kidney, PGE_2 and PGA_1 both reduce the disturbances caused by the stenosis, with the exception that renin release is further activated (Varkarakis, Szolnoky and Murphy, 1975). The rise in intrarenal prostaglandin concentrations in the ischaemic kidneys of our dogs was therefore most likely beneficial to the function of this kidney.

The selective rise in PGE seen in the dog Acorn, 1 day after the loss of 50% of her blood volume, supports the findings of Collier, Herman and Vane (1973), that hypotensive shock causes the release of renal prostaglandins. According to Johnston and Selkurt (1976), however, there is not a release of renal PGE after the haemorrhage

but only after reinfusion.

Gross et al (1976a) have shown that the changes in renal venous PGE in dog after prolonged renal ischaemia, parallel the erythropoietic activity and that indomethacin abolishes the activity of both. In addition, PGA_2 and E_2 but not $F_{2\alpha}$ when given to mice and canine kidneys stimulated erythropoiesis (Gross, Brookins, Fink and Fisher, 1976b).

In conclusion, in the two-kidney Goldblatt dog there is a transient rise in prostaglandin production by the untouched kidney, probably in response to the fluid retention, resulting in diuresis. There is also a possibility that the prostaglandins (possibly of the A series) maintain the distribution of the body fluids, thus helping to contain the hypertension. Whilst nothing can be stated about the rate of synthesis in the clamped kidney, the very early transient rise in renal venous PGE concentrations of this kidney, probably mirrors a raised intrarenal concentration, which will protect the kidney against the renal artery stenosis.

SECTION II

RENAL PROSTAGLANDINS IN EXPERIMENTAL RENAL HYPERTENSION IN RATS

METHODS

One hundred and thirty-six male and female Wistar rats, of approximately similar size and fed on a standard laboratory diet, with water ad lib, were divided into seven experimental groups, 30 rats serving as controls. Renal hypertension was induced by clipping the left renal artery with a small silver clip (first operation) and in some rats, clipping the right renal artery (second operation) two weeks later. The rats were killed at various stages after clipping as follows:

Group 1 - 20 rats killed one week after the first operation.

Group 2 - 20 rats killed two weeks after the first operation.

Group 3 - 20 rats killed three weeks after the first operation.

Group 4 - 20 rats killed four weeks after the first operation.

Group 5 - 20 rats killed one week after the second operation.

Group 6 - 20 rats killed two weeks after the second operation.

Group 7 - 16 rats killed ten weeks after the second operation. (The chronic hypertensive group).

The rats were weighed weekly and arterial blood pressure was measured by the indirect tail-cuff method using a Rat Blood Pressure Monitor (Huntingdon Instruments, England).

Renal Clearances

From each group, six to eight rats were used for the estimation of bilateral renal function, namely renal plasma flow (RPF) and glomerular filtration rate (GFR) and the remaining rats were used for the estimation of renal venous PG concentrations.

RPF and GFR were estimated by means of the clearances of PAH and of inulin respectively. The rats were anaesthetised with pentobarbitone (40 mg/kg i.p.) and tracheotomized. The left subclavian vein was catheterised for the infusion of the clearance solution at a rate of 0.05 ml/minute using a motor driven syringe (Braun). The clearance solution was composed of 1% inulin, 1% PAH and 2% Na_2SO_4 dissolved in 0.9% saline and buffered to pH 7.4 with bicarbonate.

Both ureters were catheterised after the method of Mercer (1971) and a ten minute urine sample was collected from each ureter 50 minutes after the infusion had begun. After heparinisation (1000 U/kg), blood samples (0.5 ml) were removed from a carotid artery catheter 50 and 60 minutes after the initiation of the infusion, for the calculation of mean plasma PAH and inulin concentrations, which we tried to hold at about 7 and 30 mg% respectively.

The blood was centrifuged in Eppendorf tubes and 0.2 ml of plasma removed and subjected to plasma protein precipitation using Somogyi reagent. The urine was carefully transferred to a volumetric flask and diluted to 100 ml. Diluted urine and protein-free plasma were analysed for inulin and PAH using a resorcinol method for the former N (1-napthyl)-ethylene diamine as a coupling reagent for the latter

and both were compared with standard solutions in a spectrophotometer (Bausch and Lomb, SP 20, USA).

The clearances of PAH and inulin were calculated and expressed in terms of body weight. The extraction ratio for PAH was not taken into account. Girndt and Ochwadt (1969) have shown that four weeks after clamping a renal artery, PAH extraction was unaltered in both the clamped and the contralateral kidneys (0.84 approximately).

Prostaglandin Estimation

Blood samples for the estimation of prostaglandins were drawn from the renal veins of anaesthetised rats after a midline laparotomy and catheterisation of the inferior vena cava. Within each group, the left and right renal venous samples were pooled separately (final volume of about 10 ml).

A known amount of deuterium labelled prostaglandin, 1 ug d_4 PGE_2 and 500 ng $PGF_{2\alpha}$ were added to each sample immediately before the plasma prostaglandins were extracted and subjected to silicic acid column chromatography. The plasma was acidified to pH 4 with hydrochloric acid and partitioned three times with two volumes of ethyl acetate. The combined ethyl acetate fractions were washed with 0.1 volumes of water and evaporated to dryness. The residue was dissolved in 20 ml 67% ethanol and washed twice with 20 ml petroleum spirit and evaporated to dryness. The extract was dissolved in 0.5 ml 30% ethyl acetate in toluene and the PGE_2 and $PGF_{2\alpha}$ were separated using silicic acid chromatography (Poyser, 1972).

The extracted PGE_2 and $PGF_{2\alpha}$ were converted to methyl ester/methoxime/TMS and methyl ester/TMS derivatives respectively using the methods of Thompson, Los and Horton (1970), and Blatchley, Donovan, Horton and Poyser (1972).

Using combined gas chromatography - mass spectrometry (GCMS), with a multiple ion detection system (Finnigan 3000D quadrupole mass spectrometer), the ratio of protium to deuterium peaks of each prostaglandin derivative was obtained and compared with a standard calibration over the range 1 to 1000 ng of protium (Hensby and Naylor, 1974). The m/e peaks for the protiated PGE_2 and $PGF_{2\alpha}$ were 295 and 423 and those used for the deuterated PGE_2 and $PGF_{2\alpha}$ were 299 and 427 respectively. The column used in the GC was 1 m long and 2 mm in bore containing 3% OV1 on silanised Supelco. The carrier gas flow was adjusted to 30 ml $minute^{-1}$ and the column temperature held at $230^{o}C$.

Apart from allowing the absolute amount of prostaglandin to be calculated by isotope dilution, the addition of the deuterated prostaglandins prior to extraction allowed the recoveries to be estimated. The recovery for PGE_2 was about 65% and that for $PGF_{2\alpha}$ was about 68% and the reported levels were corrected for recovery.

Statistical analysis of the results was performed by the Student two-tailed t test, except for the renal function results, where a one-tailed t test was used. Values are expressed as mean ± s.e. and only probabilities less than 0.05 were accepted as being statistically significant.

Table 5: Systolic Blood Pressure and Renal Venous PGE_2 and $PGF_{2\alpha}$ Concentrations

Group	N	Systolic	Diastolic	PGE_2		$PGF_{2\alpha}$	
				Left	Right	Left	Right
Control	30	126 ± 2.7	100 ± 2.6	4.0	3.8	2.7	2.2
1	20	139 ± 5.2 P < 0.05	111 ± 4.69 P < 0.05	4.4	2.7	3.7	2.1
2	20	142 ± 4.7 P < 0.01	112 ± 3.9 P < 0.02	5.6	3.6	4.3	2.7
3	20	148 ± 5.1 P < 0.001	123 ± 4.3 P < 0.001	4.6	2.6	3.2	2.0
4	20	148 ± 5.6 P < 0.001	118 ± 4.1 P < 0.001	3.2	5.0	1.2	2.9
5	2	152 ± 4.6 P < 0.001	121 ± 3.9 P < 0.001	3.0	4.5	1.6	4.4
6	20	193 ± 7.6 P < 0.001	153 ± 7.8 P < 0.001	4.7	6.2	2.7	3.1
7	16	183 ± 5.6 P < 0.001	150 ± 5.1 P < 0.001	2.9	4.5	1.9	3.3

Blood pressures are in mmHg, PGE_2 and $PGF_{2\alpha}$ concentrations are expressed in ng ml^{-1}.

Table 6: Average Organ Weights and Ratios

Group	N	Body Weight	Heart/Body Ratio	LK/Body Ratio	RK/Body Ratio
Control	30	257 ± 12	32.4 ± 0.6	35.2 ± 0.5	35.9 ± 0.6
1	20	226 ± 8	36.7 ± 1.2 P < 0.01	39.8 ± 1.8 P < 0.02	42.7 ± 2.0 P < 0.001
2	20	228 ± 15	35.4 ± 0.7 P < 0.01	32.6 ± 1.2 P < 0.05	35.5 ± 1.4 ns
3	20	248 ± 8	34.2 ± 0.5 P < 0.05	35.3 ± 1.8 ns	40.4 ± 1.2 P < 0.01
4	20	212 ± 11	38.5 ± 1.2 P < 0.001	34.8 ± 1.3 ns	41.3 ± 1.3 P < 0.001
5	20	292 ± 15	33.4 ± 1.4 ns	31.3 ± 1.1 P < 0.01	35.5 ± 1.2 ns
6	20	188 ± 13	41.5 ± 1.4 P < 0.001	31.6 ± 1.9 P < 0.05	40.1 ± 2.3 ns
7	20	187 ± 16	41.4 ± 2.1 P < 0.001	30.2 ± 2.1 P < 0.02	39.8 ± 2.7 ns

Average organ weight ratios. Ratios are expressed x 10^{-4}.

Body weights are in grams.

RESULTS

Development of Hypertension

One week after the production of renal ischaemia (groups 1 to 4), there was a significant rise in blood pressure, which reached a peak by the third week (group 3). Systolic blood pressure rose from 126 ± 2.73 mmHg to 148 ± 5.13 mmHg ($P < 0.001$) and diastolic pressure increased from 100 ± 2.55 mmHg to 123 ± 4.26 mmHg ($P < 0.001$) three weeks after clamping (group 3). See Table 5.

Cardiac hypertrophy also occurred over this period as seen by the rise in the heart/body weight ratio (Table 6). The left kidney/body weight ratio rose in group 1 rats but fell in group 2 and was not significantly different from control in later groups. The right kidney hypertrophied in each of the unilaterally clamped groups, except for group 2 (Table 6).

An inverse relationship between renal mass and the level of systemic hypertension was noted in almost all cases.

During ischaemia of the right kidney, as well as the left (groups 5, 6 and 7), systolic and diastolic pressures rose further, within the range 126/100 to 183/150 mmHg ten weeks after the second operation (the chronic hypertensive group).

The right kidney, which had previously been hypertrophied before the second operation, was now not significantly different from control. Cardiac hypertrophy was very marked in these bilaterally ischaemic rats.

Table 7: Renal Plasma Flow

Group	N	Left Kidney		Right Kidney	
		ml $min^{-1}kg^{-1}$	ml $min^{-1}g^{-1}$	ml $min^{-1}kg^{-1}$	ml $min^{-1}g^{-1}$
Control	10	8.23 ± 1.29	2.39 ± 0.39	9.12 ± 1.31	2.48 ± 0.37
1	8	6.34 ± 1.32 ns	1.57 ± 0.23 ns	10.14 ± 1.25 ns	2.62 ± 0.36 ns
2	8	4.28 ± 1.55 P < 0.05	1.32 ± 0.37 ns	8.56 ± 2.92 ns	2.69 ± 0.73 ns
3	8	7.46 ± 1.12 ns	2.11 ± 0.36 ns	9.00 ± 1.39 ns	2.52 ± 0.36 ns
4	8	7.59 ± 0.35 ns	2.07 ± 0.12 ns	10.95 ± 2.25 ns	2.62 ± 0.53 ns
5	8	5.85 ± 0.95 ns	1.83 ± 0.28 ns	7.14 ± 0.89 ns	2.01 ± 0.27 ns
6	7	4.44 ± 0.44 P < 0.025	1.48 ± 0.18 P < 0.05	3.43 ± 0.25 P < 0.025	1.14 ± 0.11 P < 0.005
7	6	5.49 ± 0.79 ns	1.86 ± 0.20 ns	6.30 ± 0.75 ns	1.92 ± 0.16 ns

RPF expressed relative to bodyweight (ml $min^{-1}kg^{-1}$) and kidney weight (ml $min^{-1}g^{-1}$).

Renal Function Changes

After unilateral renal ischaemia, RPF of the clamped kidney fell significantly two weeks after clamping (group 2), from 8.23 ± 1.28 ml min^{-1} kg^{-1} to 4.28 ± 1.55 ml min^{-1} kg^{-1} ($P < 0.05$) and subsequently rose again to control values (Table 7). The contralateral kidney showed no significant change in RPF at the time. Due to the well maintained GFR, the filtration fraction (FF) rose significantly only in the ischaemic kidneys of group 2 rats (Table 8).

On clamping the right kidney, RPF fell two weeks later in group 6 from 9.12 ± 1.31 ml min^{-1} kg^{-1} to 3.43 ± 0.25 ml min^{-1} kg^{-1} ($P < 0.025$) (Table 7). Left RPF also fell, from 8.23 ± 1.29 ml min^{-1} kg^{-1} to 4.44 ± 0.44 ml min^{-1} kg^{-1} ($P < 0.025$). This fall was also significant ($P < 0.0005$) when compared with group 4, which had undergone exactly the same procedures and time course, except for the second operation. As GFR was so well maintained, FF rose in the left kidneys of group 6. In group 7, the chronic hypertensive group, no difference was detected between either kidney and control.

PGE_2 and $PGF_{2\alpha}$ Renal Venous Concentrations

After the first operation, left renal venous PGE_2 and $PGF_{2\alpha}$ concentrations were higher than control, whereas the concentrations in the right renal venous plasma were similar or slightly lower than control, except for group 4. See Table 5, groups 1 to 4.

After the second operation, PGE_2 and $PGF_{2\alpha}$ concentrations rose in the right renal venous plasma one week later (group 5).

Table 8: Glomerular Filtration Rate

Group	N	Left Kidney		Right Kidney	
		ml $min^{-1}kg^{-1}$	ml $min^{-1}g^{-1}$	ml $min^{-1}kg^{-1}$	ml $min^{-1}g^{-1}$
Control	10	1.80 ± 0.25	0.52 ± 0.07	2.20 ± 0.28	0.59 ± 0.07
1	8	1.50 ± 0.26 ns	0.38 ± 0.03 ns	2.66 ± 0.28 ns	0.67 ± 0.06 ns
2	8	1.57 ± 0.39 ns	0.45 ± 0.11 ns	2.41 ± 0.59 ns	0.63 ± 0.05 ns
3	8	-	-	-	-
4	8	1.88 ± 0.18 ns	0.50 ± 0.04 ns	2.09 ± 0.33 ns	0.58 ± 0.04 ns
5	8	1.62 ± 0.15 ns	0.51 ± 0.05 ns	2.09 ± 0.23 ns	0.59 ± 0.07 ns
6	7	1.84 ± 0.27 ns	0.48 ± 0.05 ns	1.45 ± 0.35 ns	0.46 ± 0.10 ns
7	6	1.50 ± 0.16 ns	0.53 ± 0.06 ns	1.68 ± 0.19 ns	0.62 ± 0.11 ns

GFR expressed relative to bodyweight (ml $min^{-1}kg^{-1}$) and kidney weight (ml $min^{-1}g^{-1}$).

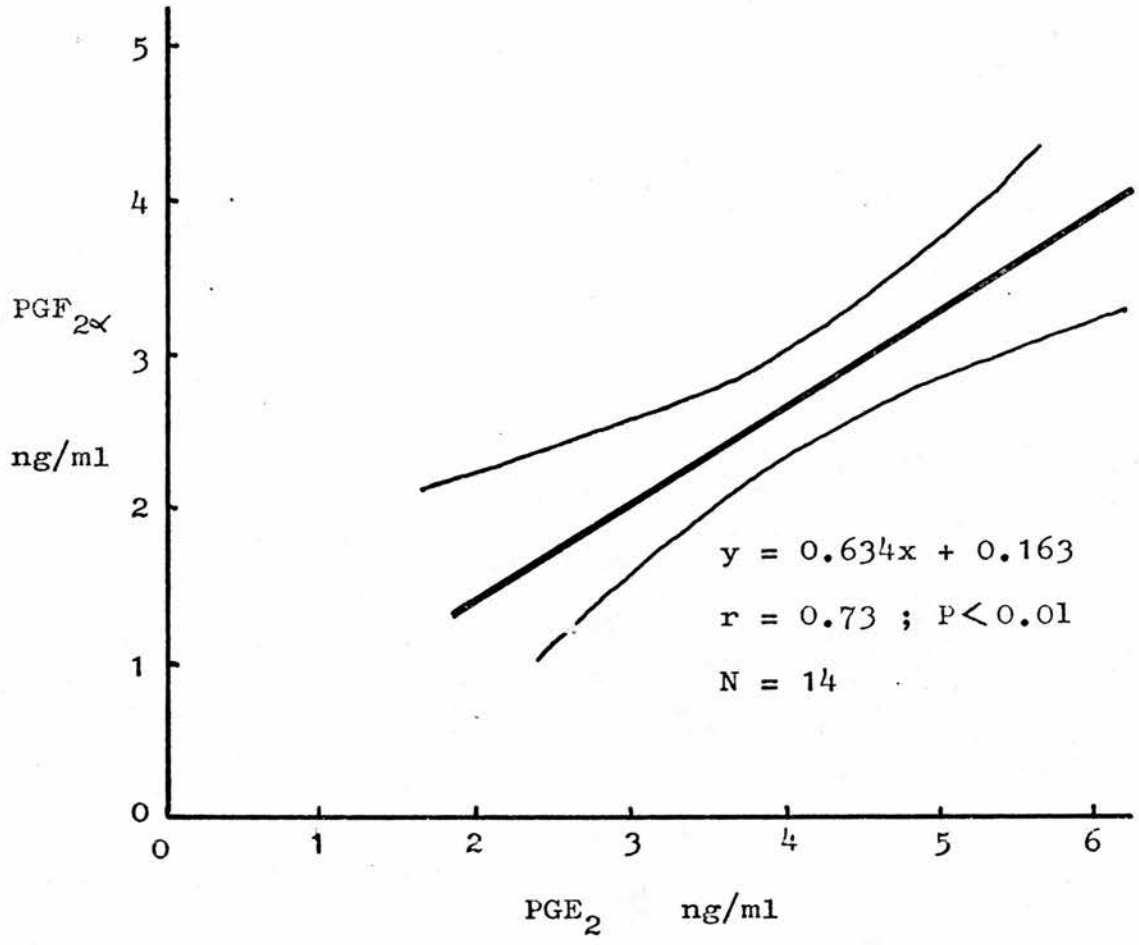

Figure 15: Correlation between PGE_2 and $PGF_{2\alpha}$ from the renal venous blood of the hypertensive rats and control rats. The 95% confidence limits are shown.

By the second week (group 6), PGE_2 had risen even more in the right renal venous plasma but $PGF_{2\alpha}$ had fallen, although still above control. Both PG s rose transiently in the left renal venous plasma. In the chronic hypertensive rats (group 7), right renal venous PGE_2 and $PGF_{2\alpha}$ concentrations were higher than control, in contrast to the left renal venous concentrations, which were lower than control.

A good correlation was found between the PGE_2 and $PGF_{2\alpha}$ concentrations, with a ratio of about 1.6 for $PGE_2/PGF_{2\alpha}$ (Figure 15).

The PGE_2 concentrations showed a very good inverse correlation with the renal plasma flow for both kidneys (Figures 16 and 17) suggesting that the concentration changes were partially if not wholly due to the flow changes. No significant correlation was seen with $PGF_{2\alpha}$.

Prostaglandin Secretion Rates

Secretion rate of each PG may be calculated from the product of the total mean RPF and the PG concentration for each group since PG s appear to be released on synthesis (Ånggård, Bohman, Griffin, Larsson and Maunsbach, 1972). This assumes that all the arterial PG s presented to the kidney are cleared in one passage because of the high activity of the metabolising enzyme, 15 hydroxy PG dehydrogenase, in the renal cortex (Ånggård, Larsson and Samuelsson, 1971). The clamped kidney shows a fall in secretion rate of PGE_2 from 8.68 ng/minute to 6.24, 5.57, 8.51 and 4.84 ng/minute in groups 1, 2, 3, and 4, respectively. $PGF_{2\alpha}$ secretion rate also fell, from 5.86 ng/minute to 5.25, 4.28, 5.92 and 1.81 ng/minute in each of the above respective groups.

See Table 9. The contralateral kidney of these groups showed no change in secretion rate of either PG. After ischaemia of the right kidney, secretion rate of both PG s began to fall in the right kidney and continued falling in the left kidney (groups 5 and 6). A subnormal secretion rate of both PG s existed in the two kidneys of the chronic hypertensive group (group 7).

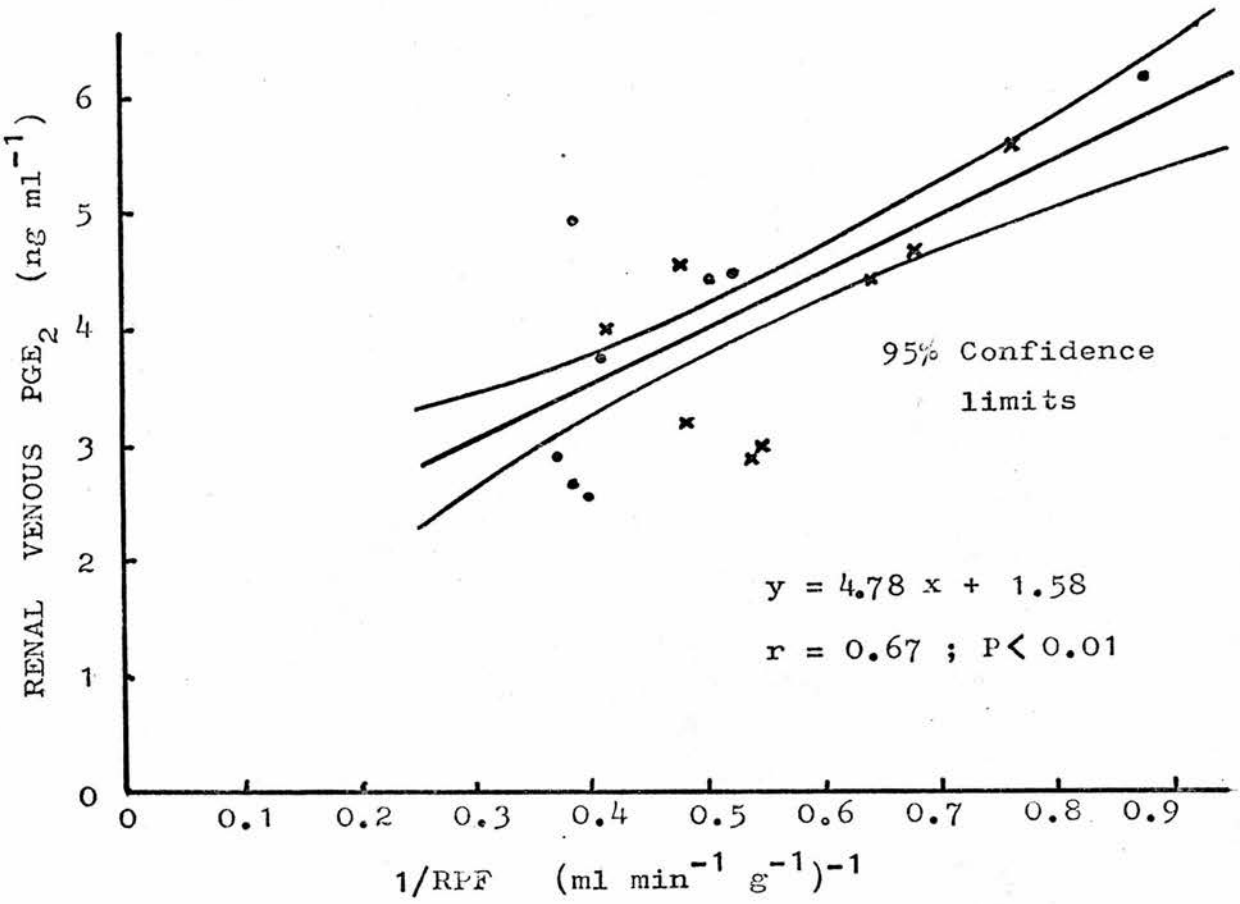

Figure 16: Relationship between the left (x) and right (•) renal venous PGE_2 and the reciprocal of the respective renal plasma flows, expressed as ml min^{-1} g^{-1}.

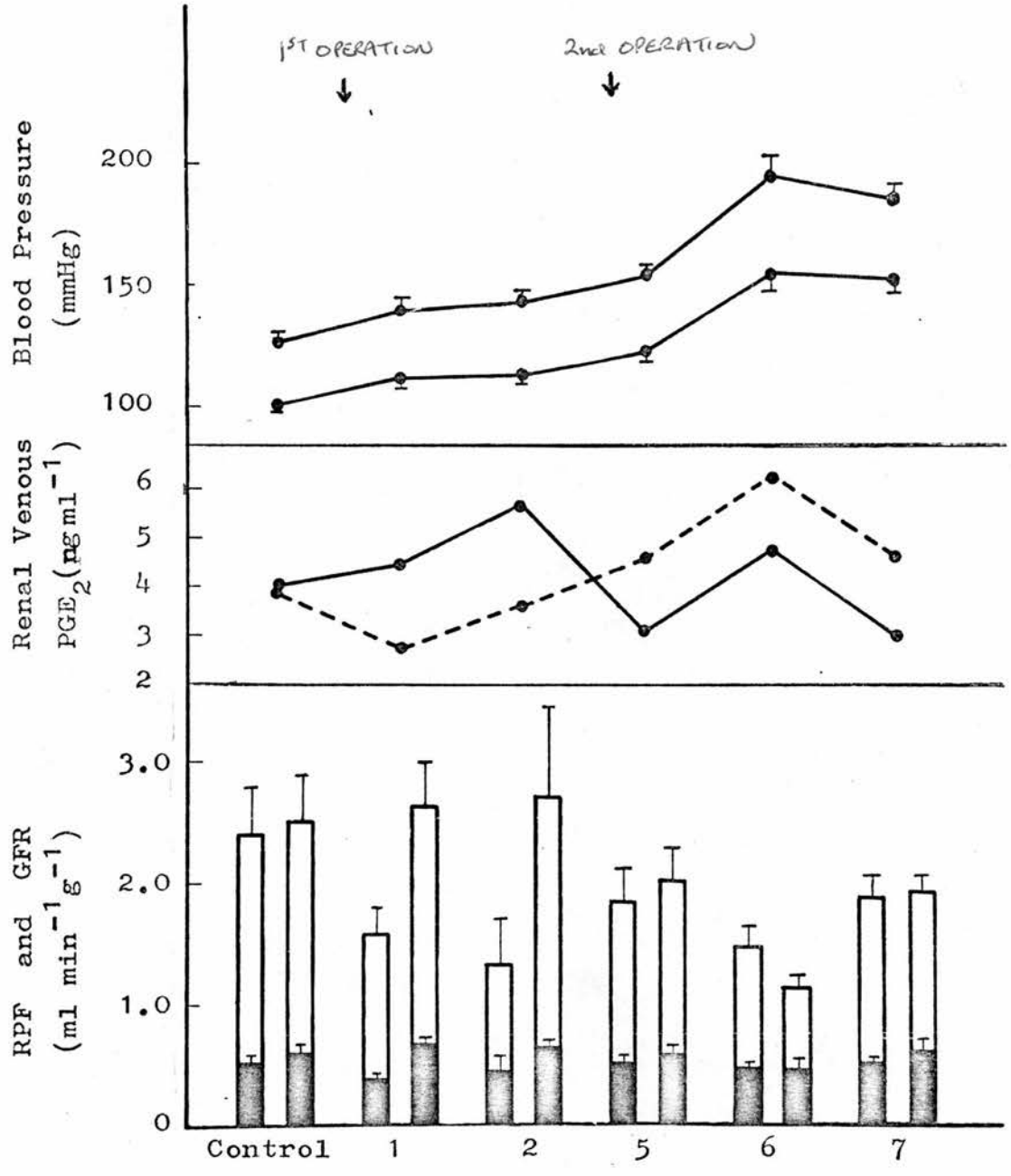

Figure 17: Systolic and diastolic blood pressures (mmHg), renal venous PGE_2 concentrations of the left (•———•) and right (•- - -•) kidneys and RPF and GFR of both kidneys (the left and right respectively in each pair) at different stages of hypertension. GFR in black bars and RPF in white bars.

Table 9: Calculated PG Secretion Rates

Group	PGE_2		$PGF_{2\alpha}$	
	Left Kidney	Right Kidney	Left Kidney	Right Kidney
Control	8.68	8.71	5.86	5.04
1	6.24	6.83	5.25	5.31
2	5.57*	8.06	4.28*	6.05
3	8.51	6.65	5.93	5.11
4	4.84	11.41	1.81	6.62
5	5.62	9.25	2.64	9.04
6	4.13*	5.51*	2.37*	2.75*
7	3.08	6.67	2.02	4.81

Calculated PG secretion rates (ng/min) obtained from the product of the PG concentration and the total mean RPF of each respective group.

* Signifies secretion rates where a significant fall in RPF was observed.

DISCUSSION

Four weeks after clamping a renal artery, the resultant hypertension was associated with normal PAH and inulin clearances in both the clamped and the contralateral kidneys of rat. However, the medullary blood flow of the contralateral kidney was elevated (Girndt and Ochwadt, 1969).

Our findings are in agreement since only a transient fall in PAH clearance was seen two weeks after clipping, but by the fourth week, the renal clearances were normal.

At variance to this, Kramer and Ochwadt (1974) report that rat kidneys clipped six to ten weeks previously had lower PAH and inulin clearances than the contralateral kidneys, but this was probably due to the dehydration and ADH infusion, which these rats underwent.

We have demonstrated that there is a rise in renal venous prostaglandin E_2 and $F_{2\alpha}$ concentrations from the clipped kidney, the other kidney showing no change. The correlation between the PGE_2 concentrations and the reciprocal of renal plasma flow (RPF) suggests that these concentration changes are not due to increased output, but are more likely due to the dilution effect of flow changes. Indeed, the calculated secretion rates of both prostaglandins in the clipped kidney falls with the progression of hypertension, whilst the secretion in the contralateral kidney does not change.

It should be realised that there are a number of assumptions made in calculating secretion rate. The kidney must clear all the arterial prostaglandins presented to it and it should release immediately on synthesis, without storing. Prostaglandins are believed to be released on synthesis (see McGiff and Nasjletti, 1973) and the kidney removes 80 to 90% of blood borne PGE_2 (Aiken and Vane, 1973) and almost all of PGA_2 in a single passage (Attallah, Payakkapan and Lee, 1974), due to the very high activity of 15 hydroxy prostaglandin dehydrogenase in the renal cortex (Ångård, Larsson and Samuelsson, 1971).

Reduced release of PGE-like material has been demonstrated in the isolated perfused kidney of the one-kidney Goldblatt rat (Leary, Ledingham and Vane, 1974) and in the incubated renal medulla from the clipped kidneys of the one and two kidney Goldblatt rats and from the Japanese spontaneously hypertensive rats (SHR), the depression being related to blood pressure (Sirvios and Gagnon, 1974). According to Pugsley, Beilin and Peto (1975) the synthetic capacity of both the clipped and the contralateral kidneys of Goldblatt rat is reduced equally by about 30% when assayed by combined GCMS.

In contrast a rise in renal immunoreactive PGE concentration is reported in the clipped kidneys of chronically hypertensive rat (Jaffe, Parker, Marshall and Needleman, 1972).

During the acute phase of hypertension an increased PGE-like concentration was seen in the clipped kidney but decreased in the chronic stages (after three months) (Somova, 1971 and 1973).

"Post-salt" hypertensive rat kidneys have elevated PGE-like concentration (Tobian and Azar, 1971) and PGA levels are greater than control in the kidneys of SHR (Zusman, Spector, Caldwell, Speroff, Schneider and Mulrow, 1973a).

Evidence from osmophilic renal interstitial cell (RICs granule counts also suggest increased prostaglandin synthesis. Decreased granule count is reported in the kidneys of SHR (Mandal, Fröhlich, Chrysant, Pfeffer and Nordquvist, 1973), "post-salt" rats (Tobian, Ishii and Duke, 1969) and in various hypertensive rat models (Muercke, Mandal and Violini, 1970). The untouched kidney shows a greater reduction than the clipped kidney and the reduction is related to the blood pressure (Ishii and Tobian, 1969).

Work in rabbits suggests that a decrease in lipid granule count is due to an increase in prostaglandin precursor turnover (Comai, Prose, Farber and Paulsmed, 1974).

The renal prostaglandins are proposed as having a natriuretic function and to protect the kidney against the vasoconstrictor/antidiuretic influence of the renin and sympathetic system (McGiff and Nasjletti, 1973 and Lonigro, Terragno, Malik and McGiff, 1973). However, in the rat kidney, unlike that of other species, PGA_2 and PGE_2 potentiate the effect of renal nerve stimulation and at higher doses actually cause vasoconstriction as does PGF_2 (Malik and McGiff, 1975). In the New Zealand genetically hypertensive rat a deficiency of 15 hydroxy prostaglandin mediated potentiation of intrarenal noradrenaline vasoconstriction. This defect might well be the cause of hypertension in these rats (Armstrong, Blackwell, Flower, McGiff, Mullane and Vane, 1976).

The inverse relationship between PGE_2 concentration and RPF, which we found might well be due to prostaglandin potentiation of vasoconstrictors.

PGE_2 is natriuretic in the rat kidney and micropuncture studies suggest that the distal tubule is the site of sodium reabsorption inhibition (Fülgraff and Meiforth, 1971) and increased blood (Papanicolaou, 1972 and 1975) and urinary output (Papanicolauo, Mountokalakis, Safar, Sostirpoulou, Bariety and Milliez, 1976) of prostaglandins are reported after fluid loading in rats. The diuresis seen after saline loading is prevented by essential fatty acid diet (Rosenthal, Simon and Silbergleit, 1974) , aspirin (Susic and Sparks, 1975) and by indomethacin (Düsing, Melder and Kramer, 1976).

However, arachidonic acid prevents excretion of a saline load in rat (Weber, Holzgreve, Stephan and Herbst, 1975).

Despite an increased BP, the blood flow and GFR of the untouched kidney are approximately normal, yet this kidney excretes twice the normal amount and two-thirds of the total sodium excreted (Kramer and Ochwadt, 1974) probably due to reduced reabsorption in the ascending loop of Henlé of the superficial nephrone (Stumpe, Lowitz and Ochwadt, 1970).

A rise in inner cortical and medullary blood flow is seen in this kidney, associated with a dissipation of the medullary solute gradient (Girndt and Ochwadt, 1969). Juxtamedullary GFR is doubled, resulting in a small increase in total GFR (Stumpe, Lowitz, and Ochwadt, 1969).

It is possible that the renal prostaglandins may account for these changes since they are natriuretic and indomethacin does reduce medullary blood flow in this species (Solez, Fox, Miller and Heptinstall, 1974). The fact that PGE_2 behaves differently on the renal vasculature in rat in comparison to other species does complicate interpretation of all the observed effects (Malik and McGiff, 1975 and Armstrong et al, 1976).

In addition we observed no change in renal venous concentration or secretion rate of either prostaglandin in the untouched kidney, but this does not remove the possibility that at certain sites within the kidney, prostaglandin concentrations may have been altered.

The increase renal venous prostaglandin concentrations in the clipped kidney, which must reflect increased intrarenal concentrations, only makes matters worse in this kidney because of potentiation of intra-renal vasoconstriction. However, the decrease in calculated secretion rate by this kidney may attenuate the effect of this mechanism.

The rat kidney does have antihypertensive properties, independent of urine loss, when introduced into the circulation of an hypertensive rat (Tobian, Schonning and Seefeldt, 1964). This property is also shared by implants of renal medulla from normal rats (Manthorpe, 1973), "post-salt" rats (Tobian and Azar, 1971) and cultures of RIC s (Muirhead, Germain, Leach, Brooks and Stephenson, 1973). The renal medulla from nomotensive rats is antihypertensive when transferred into SHR (Weiss, Manger, Praag, Hart, Hulse and Soss, 1976).

Curiously, however, the medulla from the clipped kidney is more potent than that from normal kidney and untouched kidney, exposed to the high BP has very little antihypertensive action (Manthorpe, 1973).

Prostaglandins are probably not the mediators of the renal endocrine antihypertensive property in this species although there is little doubt that prostaglandins of the E and A series are antihypertensive in this species (Somova, 1972; Wendling, Ducharme and Graham, 1972; Muirhead, Leach, Brown, Daniels and Hinman, 1967; Leach, Armstrong, Germain and Muirhead, 1971, and McQueen and Bell, 1976). Indomethacin fails to prevent the antihypertensive action of medullary implants (Manthorpe, 1975) and the extracted lipids from RIC cultures incubated in indomethacin are still effective antihypertensive material (Muirhead, Leach, Germain, Byers and Armstrong, 1974).

As far as PGE_2 is concerned, our results confirm this finding, since there was not an increase in the secretion rate in either kidney.

We did not study PGA in this project and this is the prostaglandin most likely to have an extrarenal effect, since it can escape pulmonary degradation. However, it has been show that the rat lung is not as efficient as the dog lung at removing circulating PGE since a 35% removal is reported (Papanicolaou and Meyer, 1972) as compared with about 95% in dog (Ferreira and Vane, 1967, and Robertson, 1975).

SECTION III

RENAL PROSTAGLANDINS IN RENAL BLOOD FLOW AUTOREGULATION IN THE DOG

METHODS

Ten mongrel dogs of either sex, weighing between 8 and 15.5 kg were used. After induction of anaesthesia (pentobarbitone, 30 mg/kg i.v.) the animal was tracheotomized and systemic blood pressure was measured from the left femoral artery. Temperature was held at 37^{o}C by a heating pad controlled by a thermistor rectal probe. A midline laparotomy was performed and a catheter placed in the left renal vein via a femoral vein, for renal venous blood sampling. The left ovarian or spermatic vein was ligated. Hypotonic (0.8%) saline was infused intravenously at about 2 ml/minute to ensure adequate urine flow. The animal was heparinised (1000 U/kg) and blood removed from the right carotid artery by a slowly revolving Watson Marlow MHRE pump. Part of this flow passed through a cannulating flow probe (Statham, 2.00 mm lumen) and was carried by silicone tubing through a stab wound in the abdominal wall to the catheterised left renal artery. Renal artery pressure (RAP) was measured at the tip of this catheter through a fine tube.

The remaining flow was shunted through a Starling resistance to a femoral vein. Using compressed air, with a controllable leak, the pressure in the Starling resistance box and so the perfusion pressure of the kidney, could be controlled.

Using this system the kidney could be perfused at constant pressure with the pump at a constant slow speed, thus preventing possible damage to the blood at high pump speeds.

All pressures were measured using Consolidated Electrodynamic L223 transducers and the electronically averaged pressures and renal blood flow signals were recorded on light sensitive paper (Honeywell, Visicorder, 2206). Pressure flow curves were obtained by varying RAP every five minutes, since autoregulation was complete within three minutes. After a control curve was obtained, meclofenamate (10 mg/kg i.v.) dissolved in 10 ml of 0.9% saline was injected and after 15 to 20 minutes another pressure flow curve was recorded.

10 ml samples of renal venous blood were removed at 50, 100, 150 and 200 mmHg during the determination of control pressure flow curve and at two pressures after meclofenamate in four experiments. The samples were collected in ice cold centrifuge tubes and spun at $4^{o}C$ for 25 minutes at 15,000 g. The haematocrit of each sample was noted for estimation of renal plasma flow and so prostaglandin secretion rate. The plasma was stored in a freezer for less than three weeks before the assay. The plasma was assayed for prostaglandins by radioimmunoassay as previously described in Section I.

Pressure-Flow Curve Fitting

The pressure-flow curves were plotted for each individual experiment and the best curve fitted by eye. In order to be objective, the data were also fitted by computer to a cubic expression, which describes the general shape of the autoregulation curve.

$$y = p + q\,(x - m) + r\,(x - m)^3$$

where p is the flow during autoregulation (point of inflexion)

m is the pressure at this point

q is the slope of the plateau part of the autoregulation curve

r is a constant

x is the pressure at any point

and y is the flow at any point.

Figure 18 demonstrates the results of one experiment (experiment 7) to which the cubic expression has been fitted by the computer using the method of least squares.

A straight regression line was also fitted to the experimental point. In the absence of autoregulation, the plateau gradient (q) would approximate to the regression coefficient (β) such that q/β would tend to unity. Values of q/β less than one or even negative were taken as objective evidence for autoregulation.

Values are expressed as means ± s.e. and the results were analysed using the students 't' test; probabilities less than 5% were accepted as being statistically significant.

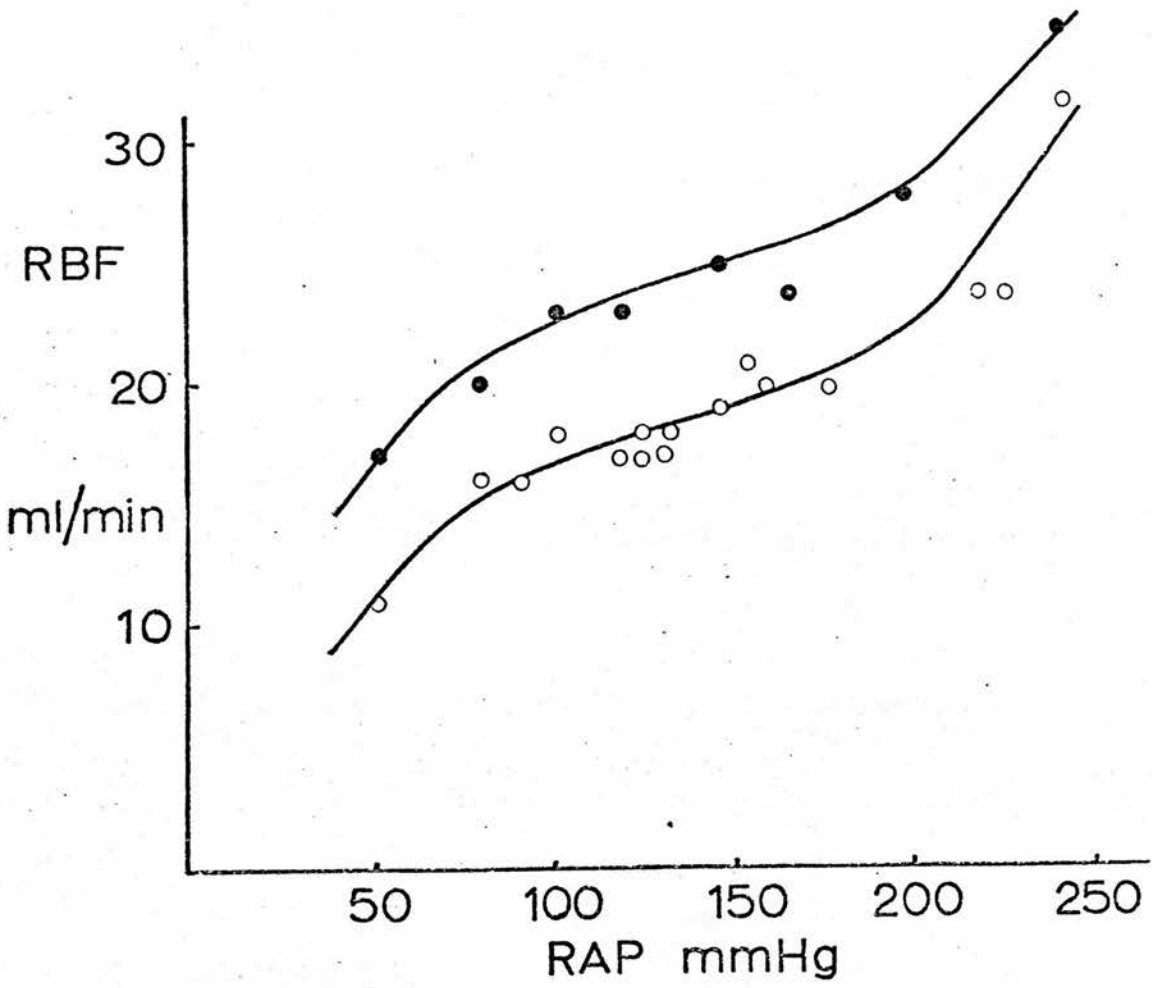

Figure 18: This figure shows the individual results of one experiment (experiment 7) before (•) and after (o) meclofenamate. The lines through the points were obtained from the cubic expression of each set of points: $y = 24.9 + 0.040\ (x - 141) + 5.26\ (x - 141)^3$ for control and $y = 18.5 + 0.037\ (x - 137) + 6.46\ (x - 137)^3$ for the meclofenamate curve.

RESULTS

Two experiments showed no autoregulation and a third lost autoregulation before completion of the control period. In the remaining seven experiments autoregulation was obtained. Mean autoregulation flow was 54 ± 13 ml/minute (n = 7) and meclofenamate (10 mg/kg i.v.) reduced it to 26 ± 6 ml/minute (n = 6, $P < 0.05$). Autoregulation was lost in one experiment after meclofenamate. The mean autoregulation flow corrected for kidney weight was 1.6 ± 0.4 ml min^{-1} per gm kidney, which is about 30% lower than that reported by Ono, Kotubin and Hashimoto (1974).

Midpoint autoregulation pressure (m) did not change after meclofenamate, 133 ± 5 mmHg before and 144 ± 10 mmHg after. The ratio q/β was always less than unity before and after meclofenamate (except in one experiment where autoregulation was lost after meclofenamate) indicating preservation of autoregulation. See Table 10 for the haemodynamic data.

Prostaglandin Concentration

There was a tendency for renal venous PGE concentrations to rise and calculated secretion rates (the product of the concentrations and their respective renal plasma flows) to fall as RAP was reduced. Using absolute values no significance could be demonstrated due to the large variation in values between experiments as is evidenced by the large standard errors. See Table 11. Individual experimental results are shown in Table M.

Table 10: Renal Haemodynamics Before and After Meclofenamate

Experiment	m	q	β	p	q/β
1A	116	0.012	0.076	29.2	0.158
1B	132	0.057	0.095	13.4	0.600
2A	133	-0.027	0.036	25.4	-0.750
2B	184	0.025	0.033	10.7	0.757
3A	120	0.016	0.180	33.9	0.089
3B	155	0.147	0.175	25.9	0.840
4A	150	0.070	0.360	73.0	0.194
4B	113	0.111	9.229	41.5	0.484
5A	145	0.059	0.538	112.7	0.110
5B	144	0.048	0.130	46.3	0.369
6A	127	0.060	0.261	80.8	0.229
6B	(42)	(0.24)	0.17	(6.97)	(1.43)
7A	141	0.040	0.080	24.9	0.560
7B	137	0.037	0.083	18.5	0.446

A - Control Values

B - Values after Meclofenamate

m = autoregulation midpoint pressure (mmHg)

q = slope of autoregulation point of curve

β = linear regression coefficient

p = is the autoregulation flow (ml/min)

q/β = is an index of autoregulation. In the absence of autoregulation this value tends to unity.

In experiment 6 autoregulation was lost after meclofenamate, the pressure-flow curve becoming virtually rectilinear. The derived parameters except β have little meaning.

By expressing the values at 50, 100 and 150 mmHg as ratios of those at 200 mmHg, a statistically significant rise in concentration of PGE was seen at the lowest pressure of 50 mmHg ($P < 0.05$) and calculated secretion rate fell ($P < 0.01$).

PGF concentrations expressed in a similar manner also rose as pressure fell, reaching statistical significance at the lowest pressure ($P < 0.05$). The rise in PGF concentration paralleled the rise in PGE concentration as shown by the excellent correlation over a wider range of concentration ($r = 0.85$, $P < 0.001$) in Figure 19. Unlike PGE secretion, PGF secretion did not change.

Meclofenamate (10 mg/kg) produced a significant fall in the calculated secretion rate of PGE at pressures of 100 and 150 mmHg from 15.65 ± 4.43 ng/min ($n = 8$) to 3.61 ± 0.61 ng/min ($n = 8$, $P < 0.05$). Renal venous plasma concentration also fell, from 315 ± 58 pg/ml to 178 ± 26 pg/ml ($P < 0.05$). PGF showed similar response, with a fall in the secretion rate from 18.93 ± 5.44 ng/min ($n = 7$) to 2.73 ± 0.27 ng/min ($n = 7$, $P < 0.01$) and in concentration from 314 ± 57 pg/ml to 134 ± 23 pg/ml ($P < 0.01$).

Table 11: Prostaglandin Concentrations (pg/ml) and Calculated Secretion Rates (ng/min) in Absolute Values and in Ratios Relative to the Values at 200 mmHg

RAP	N	PGE Concentration	PGE Secretion Rate
mmHg		pg ml^{-1}	ng min^{-1}
50	6	542 ± 222	11.31 ± 4.83
100	6	311 ± 47	10.77 ± 2.91
150	5	398 ± 127	19.47 ± 7.71
200	6	360 ± 110	15.99 ± 4.63
		Ratios	Ratios
50	6	1.40 ± 0.17*	0.65 ± 0.12†
100	6	1.11 ± 0.25	0.86 ± 0.21
150	5	1.04 ± 0.17	0.97 ± 0.25
200	6	1.00	1.00
		PGF Concentration	PGF Secretion Rate
		pg ml^{-1}	ng min^{-1}
50	4	800 ± 353	17.53 ± 7.24
100	4	237 ± 67	10.79 ± 3.84
150	4	363 ± 105	25.20 ± 9.07
200	4	368 ± 173	16.37 ± 5.62
		Ratios	Ratios
50	4	2.24 ± 0.49*	1.04 ± 0.18
100	4	1.07 ± 0.33	0.92 ± 0.30
150	4	1.33 ± 0.47	1.82 ± 0.72
200	4	1.00	1.00

* $P < 0.05$ † $P < 0.01$

DISCUSSION

Acute renal ischaemia increases the concentration of PGE-like material in the renal venous blood of the canine kidney (McGiff, Crowshaw, Terragno, Lonigro, Strand, Williamson, Lee and Ng, 1970a). Herbaczynska-Cedro and Vane (1973) confirmed this in the pump-perfused canine kidney and indomethacin (1 - 10 mg/kg i.v.) prevented both the rise in concentration of PGE-like substance and the autoregulation of blood flow. It was suggested that the kidney releases PGE_2 in response to lowered renal artery pressure (RAP) and that this mediates the fall in renal vascular resistance, resulting in the autoregulation phenomenon.

Using essentially the same approach, we still obtained autoregulation in six our of seven experiments after meclofenamate (10 mg/kg i.v.) despite a 70% fall in prostaglandin synthesis.

In the naturally perfused kidney of anaesthetised dog, prostaglandin synthetase inhibitors fail to abolish autoregulation (Owen, Ehrhart, Weidner, Scott and Haddy, 1975; Venuto, O'Dorisio, Ferris and Stein, 1975; and Anderson, Taher, Cronin, McDonald and Schrier, 1975b). The latter two groups suggest that the pump-perfused kidney may show prostaglandin dependent autoregulation due to the high resting resistance seen in this preparation. This cannot be so since we also had high resistance preparations yet still maintained autoregulation after inhibition of prostaglandin synthesis.

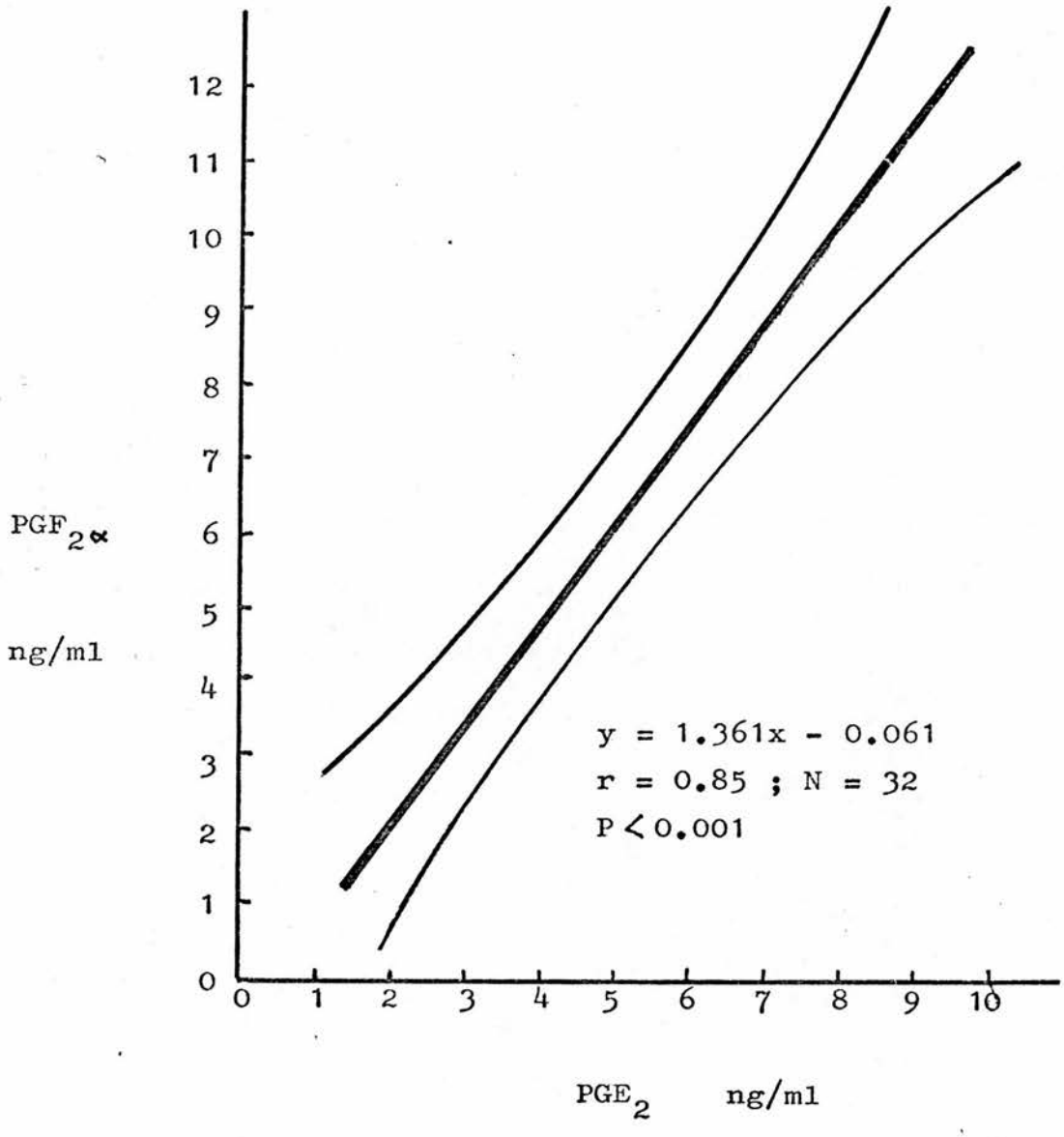

Figure 19: Correlation between the renal venous PGE_2 and the $PGF_{2\alpha}$ concentrations during the acute autoregulation experiments. The 95% confidence limits are also shown.

It is possible that despite 70% inhibition of synthesis (a figure in agreement with Venuto et al, 1975), the smaller fall in renal venous and so probably of intrarenal prostaglandin concentration, due to the concomitant fall in blood flow, may not have been sufficient to abolish autoregulation.

In agreement with Herbaczynska-Cedro and Vane (1973) and McGiff et al (1970a), we did obtain a rise in PGE concentration when flow was reduced below the autoregulation range (less than 80 mmHg). However, as the percentage flow reduction was greater than the percentage increase in PGE concentration, secretion rate must have fallen. Calculated secretion rate of PGE did fall significantly reaching statistical significance at 50 mmHg. PGF secretion rate did not change, the renal venous concentrations merely changing due to the dilution effects of the blood flow changes.

Our findings confirm the work of Beckman and Zehr (1975), who showed that a 50% reduction in renal blood flow in the dog, resulted in a 38% fall in PGE secretion rate. This is comparable to the fall of about 35%, which we obtained.

Like Beckman and Zehr, and as in our rat experiments (Section II), we assumed that the product of RPF and renal venous plasma prostaglandin concentration does give an index of secretion rate. We cannot rule out the possibility that intrarenal degradation of prostaglandins is flow dependent, such that at low flow rates, metabolism is more efficient due to the low velocity of blood in the vessels. This would have the effect of reducing the apparent secretion rate.

Absolute renal blood flow is undoubtedly dependent on prostaglandin production, since for any given pressure, we obtained a 50% reduction in flow after inhibition of synthesis. PGE_2 would appear to be the main prostaglandin determining renal blood flow (Lonigro, Itskovitz, Crowshaw and McGiff, 1973a). Infusion of prostaglandins of the E and A series (0.01 - 0.3 ug kg^{-1} min^{-1}) (Fülgraff, Brandenbusch and Heintze, 1974; and Tannenbaum, Splawinski, Oates and Nies, 1975) and arachidonic acid (1.0 - 30.0 ug kg^{-1} min^{-1}, Tannenbaum et al, 1975) into the renal artery of the dog, elevate renal blood flow and synthetase inhibition prevents the effect of arachidonate only (Tannenbaum et al, 1975).

Renal prostaglandins may also determine the distribution of renal blood flow. Indomethacin reduces inner cortical blood flow to a greater extent than outer cortical flow (Itskovitz, Stemper, Pacholczyk and McGiff, 1973). Arachidonic acid produces a rise in inner cortical flow, without much effect on outer cortical flow, whilst PGE_2 infusion increased flow to all regions of the cortex in the dog (Chang, Splawinski, Oates and Nies, 1975). In the rabbit arachidonic acid infusion also selectively increases inner cortical flow (Larsson and Ånggård, 1974).

It is, therefore, not surprising that the renal prostaglandins were suspected in renal blood flow autoregulation, especially since it has been observed that as RAP falls over the autoregulation range, there is a proportional fall in outer cortical blood flow, but inner cortical flow actually increases (McNay and Abe, 1970).

In the conscious dog, indomethacin (2 mg/kg), (Zins, 1975), meclofenamate (2 mg/kg) and the competitive synthetase inhibitor Ro 20-5720 (1 mg/kg) (Kirschenbaum and Stein, 1976) failed to affect renal blood flow or renal vascular resistance. However, no direct evidence of prostaglandin inhibition was given in these reports.

In contrast, 4 mg/kg of meclofenamate was reported to reduce renal blood flow in conscious dogs, although 10 mg/kg indomethacin did not (Swain, Hendrickx, Boettcher and Vatner, 1975).

Intrarenal blood flow distribution is not affected by prostaglandin synthetase inhibition in conscious dogs (Zins, 1975; and Kirschenbaum and Stein, 1976) but in conscious rabbits a fall in inner cortical flow is reported (Beilin and Bhattacharya, 1975).

Why should renal blood flow be resistant to the effects of synthetase inhibition in the conscious dog, yet be dependent in the anaesthetised state?

Taking precautions to prevent activation of the renin system, Satoh and Zimmerman (1975a) failed to affect renal blood flow in the anaesthetised dog with meclofenamate implying that the released angiotensin causes an abnormal stimulation of the prostaglandin system. It has been demonstrated that angiotensin causes the release of renal prostaglandins from canine kidney (Aiken and Vane, 1973). Barbiturate anaesthesia causes renin release and angiotensin production, which has been shown to reduce renal blood flow (Burger, Hopkins, Tulloch and Hollenburg, 1976). This would raise renal prostaglandin concentrations by two mechanisms, namely by angiotensin induced release and by the dilution effect of flow reduction.

Additional factors may be present in the pump-perfused kidney. The mere introduction of the superfusion bioassay system as used by Herbaczynska-Cedro and Vane (1973) has been shown to elevate arterial and renal venous PGE concentrations, possibly through kinin formation (Satoh and Zimmerman, 1976a). Under such circumstances, the renal blood flow becomes sensitive to prostaglandin synthetase inhibition. It is possible that the tubing used in the pump circuit to perfuse the kidney may produce a similar effect. The high resting resistance to flow seen in the pump-perfused kidney will also raise renal venous prostaglandin concentration through a dilution effect.

In seven conscious dogs we obtained resting renal venous PGE concentrations of 191 ± 25 pg/ml (n = 10), significantly lower than the levels obtained in the acute studies at 100 and 150 mmHg, 361 ± 63 pg/ml (n = 10, $P < 0.05$).

In conclusion, renal prostaglandins do not appear to mediate renal blood flow autoregulation, but may play a role in determining absolute flow and intrarenal flow distribution at least in the anaesthetised dog.

GENERAL DISCUSSION

In the canine kidney, by virtue of their vasodilator and natriuretic actions, the renal prostaglandins may play a role in the control of long term fluid and electrolyte balance and in protecting the kidney against the detrimental effects of renal artery stenosis upon renal function. Renal PGE is unlikely to mediate the antihypertensive role of the kidney because of a return of the raised PGE, yet blood pressure remained elevated. Moreover PGE can not circulate due to the pulmonary degradation mechanism. Elevated PGA levels, which were seen in the few dogs examined, may play a role in the control and distribution of extracellular fluid during the period of fluid retention.

In renal blood flow autoregulation in the dog, prostaglandins can not be assigned a role other than in the control of absolute renal blood flow. However, this is probably only an artefact of the anaesthesia since in the conscious dog blood flow and intrarenal blood flow distribution are unaffected by inhibition of prostaglandin synthetase, which is further evidence for renal blood flow autoregulation being mediated by a mechanism independent of prostaglandins.

Interpretation of the consequences of prostaglandin changes in the kidneys of rats is complicated by the discovery that prostaglandins are still natriuretic but potentiate vasoconstrictors and at higher doses cause intrarenal vasoconstriction by themselves in this species. The observed decrease in prostaglandin synthesis in the clipped kidney probably results in attenuation of the vasoconstrictor effects of angiotensin. The absence of a rise in PGE synthesis in

either kidney strengthens the belief that the antihypertensive action of the kidney in this species is not due to PGE.

One problem in interpreting the consequences of changes in renal venous prostaglandin concentrations in terms of effects on renal function, is that there are at least three intrarenal sites of prostaglandin synthesis, which may respond differently to the same stimulus. These sites are:-

a) the sympathetic nerve endings, mainly in the cortex, where the prostaglandins probably modulate sympathetic nerve activity (Frame and Hedqvist, 1975).

b) the medullary renal interstitial cells (RIC s), which are rich in precursors and can synthesise prostaglandins (Muirhead et al, 1973) and may play a role in sodium excretion.

c) the collecting duct cells, which according to Janszen and Nugteren (1972) are even more active than RIC s at prostaglandin synthesis and possibly account for the anti-vasopressin effects.

It is possible that this may explain why the actions of endogenous prostaglandins cannot always be simulated by administration of exogenous prostaglandins.

In evaluating changes in renal venous prostaglandin concentrations in terms of intrarenal effects it must be realised that the concentrations locally at the site of action, rather than renal output per se, is of importance. In examining the renal prostaglandin releases in terms of possible extrarenal roles, the actual rate of output is of importance as is the stability in the circulation, particularly in the pulmonary circuit.

This study has confirmed recent work that the antihypertensive action of the kidney in rat is not due to prostaglandins and that autoregulation of renal blood flow in the dog kidney is also due to a mechanism other than prostaglandins.

The roles of prostaglandins in the hypertensive dog was only speculated and it is in this system that further investigations would be of interest.

Tables A-M

In the following tables, in addition to those abbreviations on page iii, there are the following:-

SBP	systolic blood pressure in mmHg
DBP	diastolic blood pressure in mmHg
mBP	mean blood pressure in mmHg
BV	blood volume in ml/kg
PV/IFV	ratio of plasma to interstitial fluid volume
Na+	plasma sodium concentration in mEq/l
K+	plasma potassium concentration in mEq/l
BW	body weights in kilograms
RV	renal venous
A	arterial
CV	central venous
bs	below sensitivity of detection

Fluid volumes are expressed in ml/kg and Prostaglandins in pg/ml.

The observation days are in the left hand columns, where 'C' represents control values.

Table A: 'Jolly'

	SBP	DBP	mBP	HR	CVP
C	107	84	116	57	3.2
C	143	82	107	111	2.6
2	191	122	147	87	3.2
4	195	124	151	75	3.8
7	200	128	156	78	4.2
9	183	114	136	72	4.2

Kidney Weights:

Clamped 59.5 gm

Untouched 65.6 gm

	PV	BV	Ht	ECFV	IFV	PV/IFV	Na^+	K^+	BW
C	61.6	104	41	285	223	0.275	160	4.5	16.5
C	68.2	109	38	360	292	0.234	151	4.12	16.5
2	72.0	112	36	425	353	0.204	162	4.20	16.5
4	64.0	102	37	331	267	0.240	150	4.24	16.4
7	65.8	106	38	194	228	0.288	133	3.88	16.4
11	59.7	90	34	355	295	0.202	138	3.53	16.8

	PGE_2		$PGF_{2\alpha}$		PGA_2		PGB_2	
	RV	A	RV	A	RV	A	RV	A
C	130	180	200	200	600	540	420	630
2	230	190	445	240	570	bs	665	510
5	303	310	510	230	330	bs	690	890
7	210	160	120	190	bs	bs	1035	730
10	130	835	120	340	490	940	455	1820

Table B: 'Melody'

	SBP	DBP	mBP	HR	CVP
C	171	114	125	94	3.1
C	155	90	107	94	1.9
1	168	114	136	125	3.8
2	211	132	154	99	1.5
4	211	139	150	110	2.3
7	207	129	139	110	5.0
11	196	125	168	115	-

Kidney Weights:

Clamped 86.43 gm

Untouched 84.90 gm

	PV	BV	Ht	ECFV	IFV	PV/IFV	Na^+	K^+	BW
C	65.0	107	39	342	277	0.236	150	3.25	19.0
C	58.7	96	39	390	332	0.177	141	3.00	19.0
2	61.4	102	40	359	301	0.204	144	3.42	18.5
4	64.3	105	39	357	292	0.220	144	3.43	18.5
7	66.7	109	39	322	256	0.261	117	3.00	18.0
11	60.0	98	39	400	341	0.175	144	3.91	18.5

	PGE_2		$PGF_{2\alpha}$	
	RV	A	RV	A
C	129	1040	175	515
C	1370	-	2950	-
2	372	220	240	bs
5	973	180	440	420
7	415	1490	188	450
10	132	1440	bs	210

Table C 'Mandoline'

	SBP	DBP	mBP	HR	CVP
C	186	114	144	89	4.1
C	180	120	146	93	-
1	191	134	154	93	-
2	183	123	157	72	8.2
4	191	129	151	72	5.2
7	191	134	153	89	-
11	191	134	156	82	5.3
18	197	134	159	96	3.7

Kidney Weights:

Clamped 51.3 gm

Untouched 82.1 gm

	PV	BV	Ht	ECFV	IFV	PV/IFV	Na^+	K^+	BW
C	70.2	111	37	312	242	0.290	144	3.80	24.6
2	76.0	119	36	308	232	0.328	157	4.24	25.0
4	78.0	132	41	347	269	0.290	151	4.20	25.0
7	66.3	116	43	316	250	0.265	150	4.12	25.0
18	73.6	123	40	295	221	0.333	153	4.05	26.0

	PGE_2		$PGF_{2\alpha}$	
	RV	A	RV	A
C	210	174	251	181
2	490	818	260	950
5	735	880	480	240
7	510	800	525	508
10	610	620	325	190
14	412	830	215	270
21	380	-	200	-

Table D: 'Sadie'

	SBP	DBP	mBP	HR	CVP
C	178	109	120	86	3.9
3	204	136	164	78	8.3
5	169	108	135	68	13.9
7	170	108	132	63	9.1
10	168	104	132	73	-
14	178	113	144	83	-
17	162	109	135	73	6.9
19	176	115	138	73	-
*1	171	112	141	94	3.1
*4	200	121	150	89	-

Kidney Weights:

Clamped 39.55 gm

Untouched 71.75 gm

* Days after unclamping

	PV	BV	Ht	ECFV	IFV	PV/IFV	Na^+	K^+	BW
C	58.2	87	33	313	255	0.228	142	4.25	19.0
4	81.2	117	31	355	273	0.297	142	4.07	19.0
7	77.7	112	31	418	340	0.228	150	3.82	19.0
11	79.7	114	30	322	243	0.251	143	4.11	18.0
18	75.5	108	29	369	293	0.258	146	3.75	17.5
28	92.8	129	28	436	343	0.270	152	3.83	17.6

	PGE_2		$PGF_{2\alpha}$		PGA_2		PGB_2	
	RV	A	RV	A	RV	A	RV	A
C	228	2140	107	475	1415	1860	2730	2510
1	535	487	279	352	1560	1960	2345	4000
3	504	540	242	117	2820	2440	4420	4275
5	292	282	260	264	1390	1540	2400	3345
7	256	327	160	295	2790	2405	2330	4000
12	233	196	203	124	1770	1465	4510	2730
23	486	173	bs	172	2300	1820	3455	high

Table E: 'Flora'

	SBP	DBP	mBP	HR	CVP
C	175	104	129	115	3.9
C	182	104	129	99	2.1
C	168	93	125	89	2.1
1	193	125	161	120	4.6
2	211	132	168	81	4.6
3	218	143	164	110	4.6
4	236	150	175	110	3.9
7	179	114	136	125	4.6
9	188	127	146	89	-
11	167	111	133	94	-
14	178	119	152	130	-
17	181	119	141	110	-

Kidney Weights:

Clamped 55.5 gm

Untouched 89.5 gm

	PV	BV	Ht	ECFV	IFV	PV/IFV	Na^+	K^+	BW
C	68.2	108	37	312	244	0.278	121	2.80	23.0
C	63.4	101	37	302	239	0.265	128	3.49	23.0
2	57.8	95	39	299	241	0.240	142	3.73	22.8
4	51.7	83	38	297	245	0.211	147	3.84	22.6
7	61.6	99	38	293	231	0.266	152	3.82	21.5
11	70.6	114	38	336	266	0.266	132	3.45	20.5

	PGE_2		$PGF_{2\alpha}$		PGA_2		PGB_2	
	RV	A	RV	A	RV	A	RV	A
C	110	193	190	170	1500	2950	565	565
2	114	82	140	132	1790	1705	420	520
5	114	122	84	140	10400	8520	2705	2410
7	90	102	112	112	4940	6750	1825	2320
10	94	-	112	-	3480	-	1770	-
14	100	136	bs	126	9320	11130	1410	3425
17	56	158	bs	130	2055	3235	1335	1205

Table F: 'Apricot'

	SBP	DBP	mBP	HR	CVP
C	150	93	121	73	1.8
C	150	96	118	89	1.5
1	164	93	118	92	2.6
2	167	104	137	73	7.4
4	170	115	141	-	5.9
11	174	115	141	63	6.7
14	168	104	129	73	8.6

Kidney Weights:

Clamped 42.6 gm

Untouched 52.5 gm

	PV	BV	Ht	ECFV	IFV	PV/IFV	Na^+	K^+	BW
C	-	-	44	286	-	-	163	3.65	18.7
C	67.4	118	43	357	289	0.233	150	3.43	17.4
2	74.3	128	42	314	240	0.309	148	3.51	18.1
11	53.5	104	39	294	231	0.275	174	4.62	19.2
23	82.1	116	29	400	318	0.258	148	3.88	18.8

	PGE_2		$PGF_{2\alpha}$		PGA_2		PGB_2	
	RV	A	RV	A	RV	A	RV	A
C	127	260	90	263	680	820	145	395
C	130	87	190	130	820	870	260	215
2	113	220	105	250	1050	1955	710	500
5	130	135	123	145	955	1045	280	375
10	150	100	115	187	690	730	255	170
14	123	87	160	123	800	800	135	165
23	120	90	123	157	bs	645	135	120

Table G: 'Acorn'

	SBP	DBP	mBP	HR	CVP
C	152	104	130	104	1.9
C	170	100	126	94	4.8
1	152	100	130	104	-
2	179	107	132	99	4.1
4	178	111	133	78	4.8
7	174	115	133	83	2.3
11	178	119	137	63	3.7
14	-	-	125	-	-
18	161	100	121	63	2.2

Kidney Weights:

Clamped 49.2 gm

Untouched 58.4 gm

	PV	BV	Ht	ECFV	IFV	PV/IFV	Na^+	K^+	BW
C	75.8	135	44	276	200	0.378	142	3.5	20.4
C	68.3	117	44	323	258	0.253	143	3.4	20.4
2	78.4	107	27	367	288	0.272	144	4.0	20.0
4	77.5	105	26	401	323	0.240	117	3.0	20.6
7	-	-	30	295	-	-	147	4.0	20.6
11	85.7	125	31	314	228	0.375	139	4.4	20.6
18	122.0	194	36	308	187	0.656	139	3.7	20.8

	PGE_2		$PGF_{2\alpha}$	
	RV	A	RV	A
C	210	190	100	85
C	155	1709	120	370
2	400	265	122	bs
5	155	96	119	119
7	110	120	170	115
10	180	310	180	320
14	135	135	bs	105

Table H: 'Trinket'

	SBP	DBP	mBP	HR	CVP
C	155	90	119	98	3.8
C	132	77	100	84	4.3
1	132	90	113	76	2.4
2	-	-	126	56	6.7
4	148	94	119	71	2.9
11	142	94	103	76	1.0

Kidney Weights

Clamped kidney 35.04 gm

Untouched kidney 57.40 gm

	PV	BV	Ht	ECFV	IFV	PV/IFV	Na^+	K^+	BW
C	63.4	102	38	337	234	0.270	158	4.15	17.9
C	63.5	102	38	400	297	0.213	152	3.91	17.9
C	63.4	102	38	-	-	-	-	-	17.6
2	69.2	108	36	365	257	0.269	154	3.55	17.4
4	63.6	103	38	348	227	0.332	145	3.67	17.3
11	63.4	102	38	338	272	0.234	185	5.17	16.7

	PGE_2		$PGF_{2\alpha}$	
	RV	A	RV	A
C	156	183	bs	76
C	214	214	106	80
2	352	178	360	86
5	311	289	280	176
8	3950	1700	340	480
10	285	2200	296	636

Table I: 'Whisper'

	SBP	DBP	mBP	HR	CVP
C	152	100	119	76	3.8
C	148	100	119	76	5.2
1	171	113	139	71	6.7
2	168	110	126	62	-
4	158	100	126	62	6.7
7	161	103	129	71	5.2
11	174	116	139	62	5.2
14	190	132	161	67	-
16	155	110	129	64	1.4
*4	155	110	129	64	0.23
*5	146	106	127	116	0.26

Clamped Kidney 44.0 gm

Untouched Kidney 50.4 gm

* Days post-indomethacin (days 22 and 23 post clamp respectively).

	PV	BV	Ht	ECFV	IFV	PV/IFV	Na^+	K^+	BW
C	69.9	113	38	296	226	0.310	150	4.33	18.2
C	64.3	107	40	356	291	0.221	160	4.23	17.5
2	66.9	111	40	344	278	0.241	157	3.43	18.0
4	69.9	95	38	386	318	0.221	148	3.69	18.1
11	69.9	113	38	304	234	0.300	166	3.83	18.3

Table J: 'Topaz'

	SBP	DBP	mBP	HR	CVP
C	167	79	112	93	5.00
C	171	79	100	72	3.48
1	171	88	110	83	2.27
2	163	90	108	74	1.96
4	167	92	112	86	1.59
7	175	94	114	84	3.18
10	184	88	108	64	2.50

	PV	BV	HT	ECFV	IFV	PV/IFV	Na^+	K^+	BW
C	71.8	116	38	336	264	0.272	138	2.62	23.7
C	71.5	115	38	335	264	0.271	152	3.80	24.1
2	68.8	113	39	342	273	0.252	155	3.60	24.1
4	75.2	121	38	357	236	0.319	157	3.83	24.3

Table K: 'Joyless'

	SBP	DBP	mBP	HR	CVP
C	136	88	112	83	4.0
C	162	96	119	90	3.5
1	154	92	108	89	1.5
2	142	85	108	84	2.7
4	131	73	100	83	1.2
7	154	92	123	89	2.7
*3	142	92	108	-	3.1

Kidney Weights:

Clamped 66.34 gm

Untouched 77.45 gm

* Days post-indomethacin (100 mg/day).

	PV	BV	Ht	ECFV	IFV	PV/IFV	Na^+	K^+	BW
C	63.2	107	41	333	269	0.235	150	4.3	23.4
C	68.9	123	39	357	288	0.289	180	5.21	23.4
2	75.9	120	37	335	259	0.293	155	3.56	23.0

	PGE_2			$PGF_{2\alpha}$		
	RV	A	CV	RV	A	CV
C	245	330	-	180	198	-
C	350	270	-	165	200	-
Pre-Indomethacin			515	-	-	216
Post-Indomethacin			210	-	-	160

Table L: 'Jigsaw'

Kidney Weights: Clamped 59.52 gm

Untouched 65.57 gm

	PV	BV	Ht	ECFV	IFV	PV/IFV	Na^+	K^+	BW
C	66.3	111	40	351	285	0.233	155	3.46	18.9
C	63.6	108	41	352	288	0.221	162	3.84	18.9
C	66.2	110	40	354	288	0.230	161	4.18	18.9

	PGE_2		$PGF_{2\alpha}$	
	RV	A	RV	A
C	380	160	236	240
C	650	3675	276	242
C	315	-	260	-

Table M: PGE and PGF Renal Venous Plasma Concentrations and Secretion Rates at Different Renal Artery Perfusion Pressures

RAP						
(mmHg)	PGE Concentration (pg/ml)					
50	290	1630	460	370	350	150
100	390	316	480	250	280	150
150	200	730	680	240	140	-
200	180	880	420	240	220	220
	PGE Secretion Rate (ng/min)					
50	2.91	34.23	8.74	9.99	10.40	1.5
100	7.16	6.95	23.04	12.75	12.32	2.4
150	3.40	28.49	44.88	14.40	6.16	-
200	4.25	32.56	23.10	20.64	11.00	4.4
	PGF Concentration (pg/ml)					
50	-	1790	380	809	220	-
100	-	102	290	306	150	-
150	-	400	612	340	100	-
200	-	880	240	220	130	-
	PGF Secretion Rate (ng/min)					
50	-	37.59	7.22	18.72	6.60	-
100	-	2.24	18.72	15.61	6.60	-
150	-	15.60	40.39	40.39	4.40	-
200	-	32.56	13.20	13.20	6.80	-

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"Renal Blood Flow Autoregulation and Renal Venous Prostaglandins in the Pump-Perfused Canine Kidney (In Situ)." Dighe, K.K., Hall, Janet C., Smith, G.W. and Ungar, A. Br. J. Pharmac. (1977). In press.

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REFERENCES

AIKEN, J.W. and VANE, J.R. (1973).
J. Pharm. Exp. Ther., 184, 678 - 687.

ANDERSON, R.J., BERL, T., McDONALD, K.M. and SCHRIER, R.W. (1975).
J. Clin. Invest., 56, 420 - 426.

ANDERSON, R.J., TAHER, M.S., CRONIN, R.E., McDONALD, K.M. and SCHRIER, R.W. (1975).
Am. J. Physiol., 229, 731 - 736.

ÅNGGÅRD, E., LARSSON, C. and SAMUELSSON, B. (1971).
Acta Physiol. Scand., 81, 396 - 404.

ÅNGGÅRD, E., BOHMAN, S.O., GRIFFIN, J.E. III, LARSSON, C. and MAUNSBACH, A.B. (1972).
Acta Physiol. Scand., 84, 231 - 246.

ÅNGGÅRD, E., LARSSON, C. and WEBER, P. (1976).
Adv. in Prostaglandins and Thromboxanes Research. Vol. 2.
Raven press, New York. pp 587 - 594.

ARMSTRONG, J.M., BLACKWELL, G.J., FLOWER, R.J., McGIFF, J.C., MULLANE, K.M. and VANE, J.R. (1976).
Nature, 260, 582 - 586.

ASHWORTH, C.T., MUIRHEAD, E.E., THOMAS, O.F. and HILL, J.M. (1943).
Am. J. Physiol., 139, 255 - 260.

ATTALLAH, A.A., PAYAKKAPAN, W. and LEE, J.B. (1974).

Life Sci., 14, 1521 - 1534.

ATTALLAH, A.A., DUCHESNE, M.J. and LEE, J.B. (1975).

Life Sci., 16, 1743 - 1752.

BECKMAN, M.L. and ZEHR, J.E. (1975).

Prostaglandins, 9, 721 - 736.

BEILIN, L.J. and BHATTACHYRA, J. (1975).

J. Physiol., 256, 9P - 10P.

BIANCHI, G., TENCONI, L.T. and LUCCA, R. (1970).

Clin. Sci., 38, 741 - 766.

BIANCHI, G., BALDOLI, E., LUCCA, R. and BARBIN, P. (1972).

Clin. Sci., 42, 651 - 664.

BLATCHLEY, F.R., DONOVAN, B.T., HORTON, E.W. and POYSER, N.L. (1972).

J. Physiol., 223, 69 - 88.

BROWN, T., DAVIS, J.O. and OLICHNEY, M.J. (1966).

Circ. Res., 18, 475 - 483.

BOUNOUS, G. and SCHUMACKER, H.B. (1962).

Surg. Gynecol. Obstet., 144, 415 - 425.

BUMPUS, F.M., SEN, S., SMEBY, R.R., SWEET, C., FERRARIO, C.M. and KHOSLA, M.C. (1973).

Circ. Res., 32, 33, i, 150 - 158.

BURGER, B.M., HOPKINS, T., TULLOCH, A. and HOLLENBURG, N.K. (1976).
Circ. Res., 38, 196 - 202.

CARAVAGGI, A.M., BIANCHI, G., BROWN, J.J., LEVER, A.F., MORTON, J.J., POWELL-JACKSON, J.D., ROBERTSON, J.I.S. and SEMPLE, P.F. (1976).
Circ. Res., 38, 315 - 321.

CHANG, L.C.T., SPLAWINSKI, J.A., OATES, J.A. and NIES, A.S. (1975).
Circ. Res., 36, 204 - 207.

COLINA-CHOURIO, J., McGIFF, J.C. and NASJLETTI, A. (1975).
Fed. Proc., 34, 368.

COLLIER, J.G., HERMAN, A.G. and VANE, J.R. (1973).
J. Physiol., 230, 19P.

COMAI, K., PROSE, P., FARBER, S.T. and PAULSMED, J.R. (1974).
Prostaglandins, 6, 375 - 373.

CONWAY, J. and HATTON, R. (1975).
Cardiovasc. Res., 9, 229 - 235.

CORCORAN, A.C. and PAGE, I.H. (1942).
Am. J. Physiol., 135, 361 - 371.

CROWSHAW, K., McGIFF, J.C., STRAND, J.C., LONIGRO, A.J. and TERRAGNO, N.A. (1970).
J. Pharm. Pharmacol., 22, 302 - 303.

CROWSHAW, K. (1971).

Nature, New Biology, 231, 240 - 241.

DANON, A., CHANG, L.C.T., SWEETMAN, B.J., NIES, A.S. and OATES, J.A. (1975).

Biochim. Biophys. Act, 388, 71 - 83.

DAVIS, H.A. and HORTON, E.W. (1972).

Brit. J. Pharmacol., 46, 658 - 675.

DAY, M.D. and WHITING, R.L. (1972).

J. Pharm. Pharmac., 24, 263 - 264.

DIGHE, K.K., EMSLIE, H.A., HENDERSON, L.K., RUTHERFORD, F. and SIMON, L. (1975).

Br. J. Pharmac., 55, 503 - 514.

DUCHARME, D.W., WEEKS, J.R. and MONTGOMERY, R.G. (1968).

J. Pharm. Exp. Ther., 160, 1 - 10.

DUNHAM, E.W. and ZIMMERMAN, B.G. (1970).

Am. J. Physiol., 219, 1279 -

DÜSING, R., MELDER, B. and KRAMER, M.J. (1976).

Prostaglandins, 12, 3 - 9.

FASCIOLI, J.C. (1938).

Rev. Soc. Argent. Biol., 14, 15.

FEIGEN, L.P., KLAINER, E., CHAPNICK, B.M. and KADOWITZ, P.J. (1976).
J. Pharm. Exp. Ther., 198, 457 - 463.

FERRARIO, C.M., PAGE, I.H. and McCUBBIN, J.W. (1970).
Circ. Res., 27, 799 - 810.

FERRARIO, C.M., BLUMLE, C., NADZAM, G.R. and McCUBBIN, J.W. (1971).
J. Appl. Physiol., 31, 635 - 637.

FERRARIO, C.M. and McCUBBIN, J.W. (1973).
Am. J. Physiol., 224, 102 - 109.

FERRARIO, C.M. (1974).
Am. J. Physiol., 226, 711 - 717.

FERREIRA, S.H. and VANE, J.R. (1967).
Nature, 216, 868 - 873.

FICHMAN, F.P. (1970).
Clin. Res., 18, 149.

FICHMAN, F.P. and HORTON, R. (1973).
Prostaglandins, 3, 629 - 646.

FLOWER, R.J. (1974).
Pharm. Rev., 26, 33 - 67.

FRAME, M.H. and HEDQVIST, P. (1975).
Br. J. Pharmac., 54, 189 - 196.

FRÖLICH, J.C., SWEETMAN, B.J., CARR, K., HOLLIFIELD, J.W. and OATES, J.A. (1975).

Prostaglandins, 10, 185 - 195.

FÜLGRAFF, G. and MEIFORTH, A. (1971).

Pflügers Archiv., 330, 243 - 256.

FÜLGRAFF, G. and BRANDENBUSCH, G. (1974).

Pflügers Archiv., 349, 9 - 17.

FÜLGRAFF, G., BRANDENBUSCH, G. and HEINTZE, K. (1974).

Prostglandins, 8, 21 - 30.

GIBSON, M.I. and EVANS, W.A. (1937).

J. Clin. Invest., 16, 301.

GIRNDT, J. and OCHWADT, B. (1969).

Pfügers, Archiv., 313, 30 - 42.

GOLDBLATT, H. (1937).

Ann. Intern. Med., 11, 69 - 103.

GOLDBLATT, H., KALIN, J.R. and HANZAL, R.F. (1939).

J. Exp. Med., 69, 649.

GRANTHAM, J.J. and ORLOFF, J. (1968).

J. Clin. Invest., 47, 1154 - 1161.

GROLLMAN, A., MUIRHEAD, E.E. and VANATTA, J. (1949).

Am. J. Physiol., 157, 21 - 30.

GROSS, D.M., MUSJOVIC, V.M., JUBIZ, W. and FISHER, J.W. (1976).
Proc. Soc. Exp. Biol. and Med., 151, 498 - 501.

GROSS, D.M., BROOKINS, J., FINK, G.D. and FISHER, J.W. (1976).
J. Exp. Ther., 198, 489 - 496.

GUYTON, A.C. and COLEMAN, J.G. (1969).
Circ. Res., Suppl. I, 1 - 14.

HARRIS, R.C. and AYERS, C.R. (1972).
Circ. Res., 31, 520 - 530.

HENSBY, C.N. and NAYLOR, B. (1974).
Br. J. Pharmac., 52, 148P.

HERBACZYNSKA-CEDRO, K. and VANE, J.R. (1973).
Circ. Res., 33, 428 - 436.

HERBACZYNSKA-CEDRO, K. and VANE, J.R. (1974).
Nature, 247, 492.

HORNYCH, A. (1976).
Adv. in Prostaglandins and Thromboxane Research, Vol. 2.
Raven Press, New York, pp 864 - 865.

HORTON, E.W. and JONES, R.L. (1969).
Br. J. Pharmac., 37, 705 - 722.

ITSKOVITZ, M.D., STEMPER, PACHOLCZYK, and McGIFF, J.C. (1973).
Clin. Sci. 45, 321s - 324s.

ISHII, M. and TOBIAN, L. (1969).

J. Lab. Clin. Med., 74, 47 - 52.

JAFFE, N.M., PARKER, C.W., MARSHALL, G.R. and NEEDLEMAN, P. (1972).

Biochem. and Biophys. Res. Commun., 49, 799 - 805.

JANSZEN, F.M.A. and NUGTEREN, D.M. (1971).

Histochemie,, 27, 159 - 164.

JEFFREY, R.R. and SMITH, G.W. (1977).

Br. J. Pharmac. In press. [59, 437P]

JOHNSTON, P.A. and SELKURT, L.E. (1976).

Am. J. Physiol., 230, 831 - 838.

JONES, R.L., KANE, K.A. and UNGAR, A. (1974).

Br. J. Pharmac., 51, 157 - 160.

JONES, R.L. (1972).

Br. J. Pharmac., 45, 144P - 145P.

JONES, R.L. (1975).

Br. J. Pharmac., 53, 464P.

JONES, R.L. (1976).

Adv. in Prostaglandins and Thromboxane Research, Vol. 2.

Raven Press, New York, p 905.

JUBIZ, W., TERASHIMA, R. and ANDERSON, F.L. (1976).

Adv. in Prostaglandin and Thromboxane Research. Raven Press,

New York, pp 603 - 607.

KADOWITZ, P.J., SWEET, C.S. and BRODY, M.J. (1972).
Circ. Res., 30, 31, Suppl. 2, 36 - 37.

KALISKER, A. and DYER, D.C. (1972).
Eur. J. Pharmac., 19, 305 - 309.

KIRSCHENBAUM, M.A. and STEIN, J.H. (1976).
J. Clin. Invest., 57, 517 - 521.

KOLFF, J. (1958).
Circ., 17 702 - 707.

KOTCHER, T.A. and MILLER, M.C. (1974).
Am. J. Physiol., 266, 314 - 318.

KRAMER, P. and OCHWADT, B. (1974).
Nephron, 12, 464 - 474.

LARSSON, C. and ÅNGGÄRD, E. (1973).
J. Pharm. Pharmac., 25, 653 - 654.

LARSSON, C. and ÅNGGÄRD, E. (1974).
Eur. J. Pharmac., 25, 326 - 334.

LARSSON, C., WEBER, P. and ÅNGGÄRD, E. (1974).
Eur. J. Pharmac., 28, 391 - 394.

LARSSON, C. and ÅNGGÄRD, E. (1976).
Adv. in Prostaglandins and Thromboxane Research. Raven Press,
New York, pp 953 - 954.

LAVERY, H.A., LOWE, R.D. and SCROOP, G.C. (1971).
Br. J. Pharmac., 41, 454 - 461.

LAZAR, J., ROMERO, J.C. and HOOBLER, S.W. (1971).
Am. J. Physiol., 220, 191 - 193.

LEACH, B.E., ARMSTRONG, F.B., GERMAIN, G.S. and MUIRHEAD, E.E. (1973).
J. Pharm. Exp. Ther., 185, 479 - 485.

LEARY, W.P., LEDINGHAM, J.G. and VANE, J.R. (1974).
Prostaglandins, 7, 425 - 432.

LEDINGHAM, J.M. and COHEN, R.D. (1962).
Clin. Sci., 22, 69 - 77.

LEDINGHAM, J.M. and COHEN, R.D. (1964).
Can Med. Ass. J., 90, 292 - 294.

LEDINGHAM, J.M. and PELLING, D. (1967).
Circ. Res., 20, 21, II, 187 - 199.

LEDINGHAM, J.M. and PELLING, D. (1970).
J. Physiol., 210, 233 - 251.

LEE, J.B., HICKLER, R.B., SARAVIS, C.A. and THORN, (1963).
Circ. Res., 13, 359 - 366.

LEE, J.B., McGIFF, J.C., KANNEGIESSER, H., MUDD, J.G., AYKENT, Y. and FRAWLEY, T.F. (1969).
Clin. Res., 17, 456.

LEE, J.B. (1973).

Prostaglandins, 3, 551 - 579.

LEE, J.B. and ATTALLAH, A.A. (1974).

Circ. Res., 34, Suppl. 1, 78 - 82.

LEE, J.B. and ATTALLAH, A.A. (1975).

Nephron, 15, 350 - 368.

LEE, J.B. (1976).

Adv. in Prostaglandin and Thromboxane Research, Vol. 2.

Raven Press, New York, pp 573 - 585.

LEVITT, M.F. and GAUDINO, M. (1950).

Am. J. Med., 9, 208 - 215.

LIARD, J.F. (1976).

Clin. Sci. and Mol. Med., 51, 243 - 255.

LONIGRO, A.J., ITSKOVITZ, H.D., CROWSHAW, K. and McGIFF, J.C. (1973a).

Circ. Res., 32, 712 - 717.

LONIGRO, A.J., TERRAGNO, N.A., MALIK, K.U. and McGIFF, J.C. (1973b).

Prostaglandins, 3, 595 - 606.

LUCAS, J. and FLOYER, M.A. (1974).

Clin. Sci. and Mol. Med., 47, 1 - 11.

LUPU, A.N., MAXWELL, M.H., KAUFMAN, J.J. and WHITE, F.N. (1972).

Circ. Res., 30, 567 - 574.

MALIK, K.U. and McGIFF, J.C. (1975).

Circ. Res., 36, 599 - 609.

MANDAL, A.K., FRÖHLICH, E.D., CHRYSANT, K., PFEFFER, M.A. and NORDQUVIST, J. (1973).

Circ., 48, Suppl. 4, IV - 46.

MANTHORPE, T. (1973).

Acta Path. Microbiol. Scand., 81 A, 725 - 733.

MANTHORPE, T. (1975).

Acta Path. Microbiol. Scand., 83 A, 395 - 405.

McCLATCHEY, W.M. and CARR, A.A. (1973).

Prostaglandins, 3, 229 - 235.

McGIFF, J.C., CROWSHAW, K., TERRAGNO, N.A., LONIGRO, A.J., STRAND, J.G., WILLIAMSON, M.A., LEE, J.B. and NG, K.K.F. (1970a).

Circ. Res., 27, 765 - 782.

McGIFF, J.C., CROWSHAW, K., TERRAGNO, N.A. and LONIGRO, A.J. (1970b).

Nature, 227, 1255 - 1257.

McGIFF, J.C., CROWSHAW, K., TERRAGNO, N.A. and LONIGRO, A.J. (1970c).

Circ. Res., 26, 27, I, 121 - 130.

McGIFF, J.C., TERRAGNO, N.A., MALIK, K.U. and LONIGRO, A.J. (1972).

Circ. Res., 31, 36 - 43.

McGIFF, J.C. and NASJLETTI, A. (1973).

Prostaglandins and Cyclic AMP. Academic Press, New York, pp 119 - 151.

McNAY, J.L. and ABE, Y. (1970).

Circ. Res., 27, 571 - 587.

MERCER, P.F. (1971).

Can. J. Physiol. and Pharmac., 49, 420 - 426.

MILLER, E.D., SAMUELS, A.I., HABER, E. and BARGER, A.C. (1972).

Science, 177, 1108 - 1109.

MILLER, S., SAMUELS, A.I., HABER, E. and BARGER, A.C. (1975).

Am. J. Physiol., 228, 448 - 453.

MUERCKE, R.C., MANDAL, A.K. and VIOLINI, F.I. (1970).

Circ. Res., Suppl. I, 18, 109 - 119.

MUIRHEAD, E.E., STIRMAN, J.A. and JONES, F. (1960).

J. Clin. Invest., 39, 266.

MUIRHEAD, E.E., LEACH, B.E., BROWN, G.B., DANIELS, E.G. and HINMAN, J.W. (1967).

J. Lab. Clin. Med., 70, 986.

MUIRHEAD, E.E., LEACH, B.E., DANIELS, E.D. and HINMAN, J.W. (1968).

Arch. Path., 85, 72 - 79.

MUIRHEAD, E.E., LEACH, B.E., BROOKS, B., BROWN, G.B., DANIELS, E.D. and HINMAN, J.W. (1968).

Prostaglandin Symposium of Worcester Foundation. Interscience, New York, pp 183 - 200.

MUIRHEAD, E.E., BROOKS, B., PITCOCK, J.A. and STEPHENSON, P. (1972).

J. Clin. Invest., 51, 181 - 190.

MUIRHEAD, E.E., GERMAIN, G.S., LEACH, B.E., BROOKS, B. and STEPHENSON, P. (1973).

Prostaglandins, 3, 581 - 594.

MUIRHEAD, E.E., LEACH, B.E., GERMAIN, G.S., BYERS, L.W. and ARMSTRONG, F.B. (1974).

Fed. Proc., 33, 339.

MUIRHEAD, E.E., BROOKS, B. and BROSIUS, W.L. (1976).

J. Lab. Clin. Med., 88, 578 - 583.

NAKANO, J. and COLE, B. (1969).

Am. J. Physiol., 217, 222 - 227.

NEEDLEMAN, P., KAUFFMANN, A.H., DOUGLAS, J.R., JOHNSON, L.M. and MARSHALL, G.R. (1973).

Am. J. Physiol., 224, 1415 - 1419.

NEEDLEMAN, P., DOUGLAS, J.R., JAKSCHUK, B., STOECKLER, P.B. and JOHNSON, E.M. (1974).

J. Pharm. Exp. Ther., 188, 453 - 460.

ÖLIW, E., KOVER, G., LARSSON, C. and ÅNGGÄRD, E. (1976).
Eur. J. Pharmac., 38, 95 - 100.

OLSEN, V.B. (1976).
Eur. J. Pharmac., 36, 95 - 101.

OLSEN, V.B., MAGNUSSEN, M.P. and EILERTSEN, E. (1976).
Acta Physiol. Scand., 97, 369 - 376.

ONO, H., KOKOBUN, H. and HASHIMOTO, K. (1974).
Naun-Schmied. Arch. Pharmac., 28, 201 - 207.

ORLOFF, J. and HANDLER, J.S. (1965).
Nature, 205, 397 - 398.

OSMOND, D.H. (1972).
J. Lab. Clin. Med., 80, 755 - 764.

OWEN, T.L., EHRHART, K., WEIDNER, W.J., SCOTT, J.B. and HADDY, F.J. (1975).
Proc. Soc. Exp. Biol and Med., 149, 871 - 876.

PAMNANI, M.B., SIMON, G. and OVERBECK, H.W. (1976).
Circ. Res., 39, 517 - 523.

PAPANICOLAOU, N. (1972).
Experentia, 28, 275 - 276.

PAPANICOLAOU, N. and MEYER, P. (1972).
Rev. Can. Biol., 31, 313.

PAPANICOLAOU, N. (1975).

J. Pharm. Pharmacol., 27, 704 - 706.

PAPANICOLAOU, N., MOUNTOKALAKIS, T., SAFAR, M., BARIETY, J. and MILLIEZ, P. (1976).

Experentia, 32, 1015 - 1017.

PATAK, R.V., MOORKERJEE, B.K., BENTZEL, C.J., HYSERT, P.E., BABEJ, M. and LEE, J.B. (1975).

Prostaglandins, 10, 649 - 659.

PFEFFER, M.A. and FRÖHLICH, E.D. (1973).

Circ. Res., 32, 33, 28 - 33.

POLET, H. and LEVINE, L. (1975).

J. Biol. Chem., 250, 351 - 357.

POYSER, N.L. (1972).

J. Endocrinol., 54, 147 - 159.

PUGSLEY, D.J., BEILIN, L.J. and PETO, R. (1975).

Circ. Res., 36, 37, (Suppl. I), 81 - 88.

PUGSLEY, D., BEILIN, L. and PETO, R. (1976).

Adv. in Prostaglandin and Thromboxane Research, Vol. 2. Raven Press, New York, pp 595 - 598.

ROBERTSON, R.P. (1975).

Am. J. Physiol., 228, 68 - 70.

ROMERO, J., LAZAR, J., ELKINO, M. and HOOBLER, S. (1969).
Circ., 39, 40, Suppl. 3, 173 - .

ROSENTHAL, J., SIMONE, P.G. and SILBERGLEIT, A. (1974).
Prostaglandins, 5, 435 - 440.

RUMPF, K.W., FRENZEL, S., LOWITZ, M.D. and SCHELER, F. (1975).
Prostaglandins, 10, 641 - 648.

SATOH, S. and ZIMMERMAN, B.G. (1975a).
Circ. Res., 36, 37, Suppl. I, 89 - 96.

SATOH, S. and ZIMMERMAN, B.G. (1975b).
Am. J. Physiol., 229, 640 - 645.

SCHOLKENS, B.A. and STEINBACH, R. (1975).
Arch. Int. Pharmacodyn., 214, 328 - 334.

SEN, S., SMEBY, R.R. and BUMPUS, F.M. (1968).
Am. J. Physiol., 214, 337 - 341.

SIRVIOS, P. and GAGNON, D.J. (1974).
Experentia, 30, 1418 - 1419.

SOLEZ, K., FOX, J.A., MILLER, M. and HEPTINSTALL, R.H.
Prostaglandins, 7, 91 - 98.

SOMOVA, L.I. (1971).
Nephron, 8, 575 - 583.

SOMOVA, L.I. (1972).

Comptes rendes de l'Academie Bulgare des Sciences, 25, 137 - 140.

SOMOVA, L.I. (1973).

Adv. Biosci., 9, 335 - 339.

STRANDHOY, J.W., OFT, C.E., SCHNEIDER, E.G., WILLIS, L.R., BECK, N.P., DAVIS, B.B. and KNOX, F.G. (1974).

Am. J. Physiol., 226, 1015 - 1021.

STUMPE, K.O., LOWITZ, M.D. and OCHWADT, B. (1970).

J. Clin. Invest., 49, 1200 - 1212.

SURTSIN, A. and ROLF, D. (1950).

Am. J. Physiol., 161, 483 - 488.

SUSIC, D. and SPARKS, J.C. (1975).

Prostaglandins, 10, 825 - 831.

SWAIN, J.A., HEYNDRICKX, G.R., BOETTCHER, D.H. and VATNER, S.F. (1975).

Am. J. Physiol., 229, 826 - 830.

SWEET, C.S., KADOWITZ, P.J. and BRODY, M.J. (1971).

Eur. J. Pharmac., 16, 229 - 232.

TANNENBAUM, J., SPLAWINSKI, J.A., OATES, J.A. and NIES, A.S. (1975).

Circ. Res., 36, 197 - 203.

TARAZI, R.C., DUSTAN, M.P. and FROHLICH, E.D. (1969).

Circ., 40, 357 - .

THOMPSON, C.J., LOS, M. and HORTON, E.W. (1970).
Life Sci., 9, 983 - 988.

TOBIAN, L., SCHONNING, S. and SEEFELDT, C. (1964).
Ann. Intern. Med., 60, 378 - 383.

TOBIAN, L., ISHII, M. and DUKE, M. (1969).
J. Lab. Clin. Med., 73, 309 - 319.

TOBIAN, L. and AZAR, S. (1971).
Trans. Am. Assoc. Physicians, 84, 281 - 288.

TOBIAN, L. and O'DONNELL, M. (1976).
Fed. Proc., 35, 2388 - 2392.

VANCE, V.K., ATTALLAH, A.A., PREZYNA, A. and LEE, J.B. (1973).
Prostaglandins, 3, 649 - 667.

VANKERWEGHEM, J.L., DUCOBU, J. and D'HOLLANDER, A. (1975).
Pflügers Archiv., 357, 243 - 252.

VARKARAKIS, M.J., SZOLNOKY, A. and MURPHY, G.P. (1975).
Invest. Urol., 12, 302 - 308.

VENUTO, R.G., O'DORISIO, T., FERRIS, T.F. and STEIN, J.H. (1975).
Prostaglandins, 9, 817 - 828.

WEBER, P., HOLZGREVE, H., STEPHAN, R. and HERBST, R. (1975).
Eur. J. Pharmac., 34, 299 - 304.

WEISS, R.J., MANGAR, W.M., PRAAG, D.V., HART, C.J., HULSE, M. and SOSS, G.A. (1976).

The Pharmacologist, 18, 162, 279.

WENDLING, M.G., DUCHARME, D.W. and GRAHAM, B.E. (1972).

The Pharmacologist, 15, 301.

ZIMMERMAN, B.G. (1973).

Life Sci., 13, 507 - 515.

ZINS, G.R. (1975).

Am. J. Med., 58, 14 - 24.

ZUSMAN, R.M., CALDWELL, B.V. and SPEROFF, L.S. (1972).

Prostaglandins, 2, 41 - 53.

ZUSMAN, R.M. (1973).

J. Clin. Invest., 52, 93a.

ZUSMAN, R.M., SPECTOR, D., CALDWELL, B.V., SPEROFF, L., SCHNEIDER, G. and MULROW, P.J. (1973).

J. Clin. Invest., 52, 1093 - 1098.

ZUSMAN, R.M., FORMAN, B.H., SCHNEIDER, G., CALDWELL, B.V., SPEROFF, L. and MULROW, P.J. (1973).

Clin. Sci. and Mol. Med., 45, 325s - 329s.

ERRATA

The following references have been omitted:-

DANIELS, E.G., HINMAN, J.W., LEACH, B.E. & MUIRHEAD, E.E. (1967). Nature, 215, 1298.

ELKINGTON, J.R. & TAFFEL, M. (1942). Am. J. Physiol., 138, 126-135.

McQUEEN, D. & BELL, K. (1976). Eur. J. Pharmacol., 37, 223-225.

Br. J. Pharmac. (1976), **58**, 253–259

RENAL FUNCTION AND RENAL VENOUS PROSTAGLANDIN CONCENTRATIONS DURING DIFFERENT STAGES OF EXPERIMENTAL RENAL HYPERTENSION IN THE RAT

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1 Renal hypertension was produced in rats and the changes in renal function, renal venous prostaglandin E_2 and $F_{2\alpha}$ concentrations and secretion rates were studied at various times.

2 Renal plasma flow transiently fell in the ischaemic kidney 2 weeks after clamping, whilst that of the other kidney did not change. Glomerular filtration rate remained constant in both kidneys throughout the entire study.

3 Prostaglandins E_2 and $F_{2\alpha}$ concentrations rose in the venous plasma from the ischaemic kidney, but did not change in the other kidney and appeared to be inversely related to renal plasma flow.

4 Calculated secretion rate of both prostaglandins fell in the ischaemic kidney and did not change in the other kidney.

5 Clamping the second kidney, two weeks after the first, caused a further elevation in blood pressure, a fall in renal plasma flow and a fall in prostaglandin secretion rate in both kidneys.

6 The implications of these prostaglandin changes are discussed.

Introduction

Acute renal ischaemia increases the concentration of prostaglandin E-like material in renal venous blood of the dog and this has been interpreted as a rise in output of renal prostaglandins (McGiff, Crowshaw, Terragno, Lonigro, Strand, Williamson, Lee & Ng, 1970). Such a mechanism may protect the kidney from the antinatriuretic/vasoconstrictor influence of the renin and sympathetic systems (Lonigro, Terragno, Malik & McGiff, 1973).

The contralateral renal prostaglandins have also been assigned a role. The contralateral kidney of the Goldblatt hypertensive dog has an antihypertensive function, independent of excretion (Grollman, Muirhead & Vanatta, 1949). Such a system is also present in the rat kidney, when perfused at hypertensive pressures (Tobian, Schonning & Seefeldt, 1964). Lee (1973) believes that the renal prostaglandins may play a role.

No study has yet examined renal venous prostaglandin concentrations in chronic renal ischaemia of experimental origin nor attempted to evaluate the rate of output of renal prostaglandins.

In this study we examined renal function and renal venous prostaglandin E_2 and $F_{2\alpha}$ concentrations of both kidneys of rats at various stages after clamping the left renal artery and also after clamping both renal arteries.

Methods

One hundred and thirty six male and female Wistar rats, of approximately similar size and fed on a standard laboratory diet, with water *ad lib.*, were divided into seven experimental groups, 30 rats serving as controls. Renal hypertension was induced by clipping the left renal artery with a small silver clip (first operation) and in some rats, clipping the right renal artery (second operation) two weeks later. The rats were killed at various stages after clipping as follows: group 1, 20 rats killed 1 week after the first operation; group 2, 20 rats killed 2 weeks after the first operation; group 3, 20 rats killed 3 weeks after the first operation; group 4, 20 rats killed 4 weeks after the first operation; group 5, 20 rats killed 1 week after the second operation; group 6, 20 rats killed 2 weeks after the second operation and group 7, 16 rats killed 10

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weeks after the second operation (the chronic hypertensive group).

The rats were weighed weekly and arterial blood pressure was measured by the indirect tail-cuff method using a Rat Blood Pressure Monitor (Huntingdon Instruments, England).

From each group, 6 to 8 rats were used for the estimation of bilateral renal function, namely renal plasma flow (RPF) and glomerular filtration rate (GFR) and the remaining rats were used for the estimation of renal venous prostaglandin concentrations.

RPF and GFR were estimated by means of the clearances of para-aminohippurate (PAH) and of inulin respectively. The rats were anaesthetized with pentobarbitone (40 mg/kg i.p.) and tracheotomized. The left subclavian vein was catheterized for the infusion of the clearance solution at a rate of 0.05 ml/min by means of a motor driven syringe (Braun). The clearance solution was composed of 1% inulin, 1% PAH and 2% Na_2SO_4 dissolved in 0.9% w/v NaCl solution and buffered to pH 7.4 with bicarbonate.

Both ureters were catheterized after the method of Mercer (1971) and a 10 min urine sample was collected from each ureter 50 min after the infusion had begun. After heparinization (1000 u/kg), blood samples (0.5 ml) were removed from a carotid artery catheter 50 and 60 min after the start of the infusion, for the calculation of mean plasma PAH and inulin concentrations, which we tried to hold at about 7 and 30 mg% respectively.

The blood was centrifuged in Eppendorf tubes and 0.2 ml of plasma removed and subjected to plasma protein precipitation with Somogyi reagent. The urine was carefully transferred to a volumetric flask and diluted to 100 ml. Diluted urine and protein-free plasma were analysed for inulin and PAH using a resorcinol method for the former and *N* (1-napthyl)-ethylene diamine as a coupling reagent for the latter and both were compared with standard solutions in a spectrophotometer (Bausch and Lomb, SP 20, U.S.A.).

The clearances of PAH and inulin were calculated and expressed in terms of body weight. The extraction ratio for PAH was not taken into account. Girndt & Ochwadt (1969) have shown that 4 weeks after clamping a renal artery, PAH extraction was unaltered in both the clamped and the contralateral kidneys (0.84 approx.).

Blood samples for the estimation of prostaglandins were drawn from the renal veins of anaesthetized rats after a midline laparotomy and catheterization of the inferior vena cava. Within each group, the left and right renal venous blood samples were pooled separately (final volume about 10 ml). A known amount of deuterium isotope, 1 μg d_4 prostaglandin E_2 and 500 ng d_4 prostaglandin $F_{2\alpha}$ was added to each plasma sample and the prostaglandins were extracted and subjected to silicic acid column chromatography (Poyser, 1972). Methyl ester/methoxime/trimethylsilyl and methyl ester/trimethylsilyl derivatives were made of the extracted prostaglandins E_2 and $F_{2\alpha}$ respectively (Thompson, Los & Horton, 1970; Blatchley, Donovan, Horton & Poyser, 1972). Using multiple ion detection (Finnigan 3000D mass

Table 1 Changes in blood pressure (mmHg) and prostaglandin (PG) level (ng/ml) in renal venous blood of control and renal hypertensive rats

Group	(n)	Blood pressure		PGE_2		$PGF_{2\alpha}$	
		Systolic	Diastolic	Left renal vein	Right renal vein	Left renal vein	Right renal vein
Controls	(30)	126 ± 2.73	100 ± 2.55	4.0	3.8	2.7	2.2
1	(20)	139 ± 5.17 $P<0.05$	111 ± 4.69 $P<0.05$	4.4	2.7	3.7	2.1
2	(20)	142 ± 4.71 $P<0.01$	112 ± 3.91 $P<0.02$	5.6	3.6	4.3	2.7
3	(20)	148 ± 5.13 $P<0.001$	123 ± 4.26 $P<0.001$	4.6	2.6	3.2	2.0
4	(20)	148 ± 5.62 $P<0.001$	118 ± 4.14 $P<0.001$	3.2	5.0	1.2	2.9
5	(20)	152 ± 4.57 $P<0.001$	121 ± 3.87 $P<0.001$	3.0	4.5	1.6	4.4
6	(20)	193 ± 7.61 $P<0.001$	153 ± 7.84 $P<0.001$	4.7	6.2	2.7	3.1
7	(16)	183 ± 5.55 $P<0.001$	150 ± 5.14 $P<0.001$	2.9	4.5	1.9	3.3

n is the number of rats in each group. Values are mean ± s.e. Groups 1 to 4 had clipped left kidneys. Groups 5 to 7 had both kidneys clipped.

spectrometer), the ratio of proton to deuterium peaks of the prostaglandin derivatives was obtained and compared with a standard calibration curve over the range of 1 to 1000 ng of protium. (Hensby & Naylor, 1974.)

Statistical analysis of the results was performed by Student's two-tailed t test, except for the renal function results, where a one-tailed t test was used (Dixon & Massay, 1969). Values are expressed as mean $\pm$ s.e. and only probabilities less than 0.05 were accepted as being statistically significant.

Results

Development of hypertension

One week after the production of renal ischaemia (groups 1 to 4), there was a significant rise in blood pressure, which reached a peak by the third week (group 3). Systolic blood pressure rose from 126 ± 2.73 mmHg to 148 ± 5.13 mmHg ($P < 0.001$) and diastolic pressure increased from 100 ± 2.55 mmHg to 123 ± 4.26 mmHg ($P < 0.001$) three weeks after clamping (group 3) (Table 1).

Cardiac hypertrophy also occurred over this period as seen by the rise in the heart/body weight ratio (Table 2). The left kidney/body weight ratio rose in group 1 rats but fell in group 2 and was not significantly different from control in later groups. The right kidney hypertrophied in each of the unilaterally clamped groups, except for group 2 (Table 2).

An inverse relationship between renal mass and the level of systemic hypertension was noted in almost all cases.

During ischaemia of the right kidney, as well as of the left, (groups 5, 6 and 7), systolic and diastolic pressures rose further, within the range 126/100 to 183/150 mmHg 10 weeks after the second operation (the chronic hypertensive group).

The left, earlier ischaemic kidney, was now firm and shrunken, especially in the chronic group (group 7). The right kidney, which had previously been hypertrophied before the second operation, was now not significantly different from control. Cardiac hypertrophy was very marked in these bilaterally ischaemic rats.

Renal function changes

After unilateral renal ischaemia, RPF of the clamped kidney fell significantly 2 weeks after clamping (group 2), from 8.23 ± 1.28 ml min^{-1} kg^{-1} to 4.28 ± 1.55 ml min^{-1} kg^{-1} ($P < 0.05$) and subsequently rose again to control values (Table 3). The contralateral kidney showed no significant change in RPF at this time. Due to the well maintained GFR, the filtration fraction (FF) rose significantly only in the ischaemic kidneys of group 2 rats.

On clamping the right kidney, RPF fell 2 weeks later in group 6 from 9.12 ± 1.31 ml min^{-1} kg^{-1} to 3.43 ± 0.25 ml min^{-1} kg^{-1} ($P < 0.025$) (Table 3). Left RPF also fell, from 8.23 ± 1.29 ml min^{-1} kg^{-1} to 4.44 ± 0.44 ml min^{-1} kg^{-1} ($P < 0.025$). This fall was also significant ($P < 0.0005$) when compared with group 4, which had undergone exactly the same procedures and time course, except for the second

Table 2 Average organ/body weights in control and experimental rats.

Group	(n)	*Body weight (g)*	*Heart/body weight*	*Left kidney/ body weight*	*Right kidney/ body weight*
Controls	(30)	257 ± 12	32.4 ± 0.6	35.2 ± 0.5	35.9 ± 0.6
1	(20)	226 ± 8	36.7 ± 1.2	39.8 ± 1.8	42.7 ± 2.0
			$P < 0.01$	$P < 0.02$	$P < 0.001$
2	(20)	228 ± 15	35.4 ± 0.7	32.6 ± 1.2	35.5 ± 1.4
			$P < 0.01$	$P < 0.05$	$P > 0.5$
3	(20)	248 ± 8	34.2 ± 0.5	35.3 ± 1.8	40.4 ± 1.2
			$P < 0.05$	$P > 0.5$	$P < 0.01$
4	(20)	212 ± 11	38.5 ± 1.2	34.8 ± 1.3	41.3 ± 1.3
			$P < 0.001$	$P > 0.5$	$P < 0.001$
5	(20)	292 ± 15	33.4 ± 1.4	31.3 ± 1.1	35.5 ± 1.2
			$P > 0.5$	$P < 0.01$	$P > 0.5$
6	(20)	188 ± 13	41.5 ± 1.4	31.6 ± 1.9	40.1 ± 2.3
			$P < 0.001$	$P < 0.05$	$P > 0.05$
7	(16)	187 ± 16	41.4 ± 2.1	30.2 ± 2.1	39.8 ± 2.7
			$P < 0.001$	$P < 0.02$	$P > 0.1$

Body weights are expressed in grams. Organ/body weights are expressed $\times 10^{-4}$. n is the number of rats in each group. Values are mean $\pm$ s.e. Groups 1 to 4 had clipped left kidneys. Groups 5 to 7 had both kidneys clipped.

operation. As GFR was so well maintained, FF rose in the left kidneys of group 6. In group 7, the chronic hypertensive group, no difference was detected between either kidney and control.

Prostaglandins E_2 and $F_{2\alpha}$ renal venous concentrations

After the first operation, left renal venous prostaglandin E_2 and $F_{2\alpha}$ concentrations were higher than control, whereas the concentrations in the right renal venous plasma were similar or slightly lower than control, except for group 4 (see Table 1, groups 1 to 4).

After the second operation, prostaglandins E_2 and $F_{2\alpha}$ concentrations rose in the right renal venous plasma 1 week later (group 5). By the second week (group 6), prostaglandin E_2 had risen even more in the right renal venous plasma but prostaglandin $F_{2\alpha}$ had fallen, although still above control. Both prostaglandins rose transiently in the left renal venous plasma. In the chronic hypertensive rats (group 7), right renal venous prostaglandin E_2 and $F_{2\alpha}$ concentrations were higher than control, in contrast to the left renal venous concentrations, which were lower than control.

Prostaglandin secretion rates

Secretion rate of each prostaglandin may be calculated from the product of the total mean RPF and the prostaglandin concentration for each group since prostaglandins appear to be released on synthesis (Ånggärd, Bohman, Griffin, Larsson & Maunsbach, 1972). This assumes that all the arterial prostaglandins presented to the kidney are cleared in one passage because of the high activity of the metabolizing enzyme, 15 hydroxy prostaglandin dehydrogenase, in the renal cortex (Ånggärd, Larsson & Samuelsson, 1971). The clamped kidney showed a fall in secretion rate of prostaglandin E_2 from 8.68 ng/min to 6.24, 5.57, 8.51 and 4.84 ng/min in groups 1, 2, 3 and 4 respectively. Prostaglandin $F_{2\alpha}$ secretion rate also fell, from 5.86 ng/min to 5.25, 4.28, 5.92 and 1.81 ng/min in each of the above respective groups (see Table 4). The contralateral kidney of these groups showed no change in secretion rate of either prostaglandin. After ischaemia of the right kidney, secretion rate of both prostaglandins began to fall in the right kidney and continued falling in the left kidney (groups 5, and 6). A subnormal secretion rate of both prostaglandins existed in the two kidneys of the chronic hypertensive group (group 7).

Discussion

Four weeks after clamping a renal artery, the resultant hypertension was associated with normal PAH and inulin clearances in both the clamped and contralateral kidneys of rats (Girndt & Ochwadt, 1969). However, medullary blood flow was elevated in the contralateral kidney as determined by the ^{86}Rubidium method. Our results are essentially in agreement with these findings. However, at variance with this, Kramer & Ochwadt (1974) reported that rat kidneys clamped 6 to 10 weeks previously, had lower PAH and inulin clearances in comparison with the contralateral kidney.

Table 3 Glomerular filtration rate and renal plasma flow

Group	(n)	*Glomerular filtration rate*		*Renal plasma flow*		*Filtration fraction*	
		Left kidney	*Right kidney*	*Left kidney*	*Right kidney*	*Left kidney*	*Right kidney*
Controls	(10)	1.80 ± 0.25	2.20 ± 0.28	8.23 ± 1.29	9.12 ± 1.31	0.24 ± 0.03	0.27 ± 0.05
1	(8)	1.50 ± 0.26	2.66 ± 0.28	6.34 ± 1.32	10.14 ± 1.25	0.28 ± 0.03	0.30 ± 0.04
		$P > 0.2$	$P > 0.05$	$P > 0.05$	$P > 0.2$	$P > 0.2$	$P > 0.2$
2	(8)	1.57 ± 0.39	2.41 ± 0.59	4.28 ± 1.55	8.56 ± 2.92	0.41 ± 0.07	0.38 ± 0.05
		$P > 0.2$	$P > 0.2$	$P < 0.05$	$P > 0.2$	$P > 0.05$	$P > 0.1$
3	(8)	—	—	7.46 ± 1.12	9.00 ± 1.39	—	—
				$P > 0.2$	$P > 0.2$		
4	(8)	1.88 ± 0.18	2.09 ± 0.33	7.59 ± 0.35	10.95 ± 2.25	0.21 ± 0.02	0.28 ± 0.06
		$P > 0.02$	$P > 0.2$	$P > 0.2$	$P > 0.2$	$P > 0.5$	$P > 0.5$
5	(8)	1.62 ± 0.15	2.09 ± 0.23	5.85 ± 0.95	7.14 ± 0.89	0.30 ± 0.02	0.31 ± 0.01
		$P > 0.2$	$P > 0.2$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.2$
6	(7)	1.84 ± 0.27	1.45 ± 0.35	4.44 ± 0.44	3.43 ± 0.25	0.42 ± 0.09	0.45 ± 0.14
		$P > 0.2$	$P > 0.05$	$P < 0.025$	$P < 0.025$	$P \geqslant 0.05$	$P > 0.2$
7	(6)	1.50 ± 0.16	1.68 ± 0.19	5.49 ± 0.79	6.30 ± 0.75	0.34 ± 0.06	0.37 ± 0.08
		$P > 0.2$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.1$	$P > 0.1$

Values are mean ± s.e. Clearances are expressed in ml min^{-1} kg^{-1} body weight. *n* is the number of rats in each group. Groups 1 to 4 had clipped left kidneys. Groups 5 to 7 had both kidneys clipped.

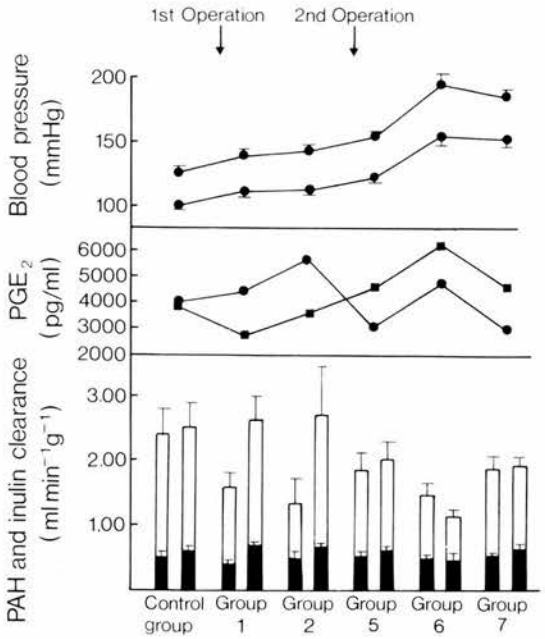

Figure 1 Systolic and diastolic blood pressures (mmHg), renal venous prostaglandin E_2 concentrations of the left (●) and right (■) kidneys and RPF (white bars) and GFR (black bars) of the left and right kidneys respectively in each pair, at different stages of hypertension. Values are mean ± s.e.

McGiff *et al.* (1970) propose that the kidney releases prostaglandin-like material in response to reduced renal blood flow caused by constricting the renal artery. Herbaczynska-Cedro & Vane (1973) obtained a rise in renal venous prostaglandin E-like material on reducing perfusion pressure and renal blood flow, in the pump perfused canine kidney and suggested that increased secretion of prostaglandin E mediated autoregulation of blood flow since indomethacin abolished autoregulation and the prostaglandin release. However, this concept may be no longer tenable in the intact, naturally perfused kidney (Owen, Ehrhart, Weidner, Haddy & Scott, 1974; Venuto, O'Dorisio, Ferris & Stein, 1975; Anderson, Taher, Cronin, McDonald & Schrier, 1975). Using essentially the same approach as Herbaczynska-Cedro & Vane, autoregulation was found to be resistant to meclofenamate (10 mg/kg, i.v.) although absolute flow fell (Dighe, Hall, Smith & Ungar, unpublished results). It remains to be seen whether rat kidney behaves in a similar manner.

Renal prostaglandins may have a natriuretic role and protect the kidney against the vasoconstrictor/antidiuretic influence of the renin and sympathetic systems (McGiff & Nasjletti, 1973; Lonigro, *et al.*, 1973). However, in the isolated kidney of the rat, unlike other species, prostaglandin E_2 potentiates renal sympathetic nerve stimulation and at higher doses causes vasoconstriction similar to that induced by prostaglandin $F_{2\alpha}$ (Malik & McGiff, 1975).

We have shown that prostaglandin E_2 and $F_{2\alpha}$ concentrations rise in the plasma from ischaemic kidneys, whereas the contralateral renal venous concentrations remain unaltered or may perhaps fall slightly. As Figure 1 shows, there is an inverse relationship between prostaglandin E_2 concentration and RPF for both kidneys. This may either be a result or a cause of the blood flow changes. Thus an increase in prostaglandin E_2 or $F_{2\alpha}$ concentrations may reduce RPF by the mechanism described by Malik & McGiff (1975) and conversely a fall in RPF may be due to a fall in prostaglandin concentration.

Alternatively, the concentration changes may be a consequence of flow changes. This is supported by the findings of Beckman & Zehr (1975) in the dog. On halving renal blood flow, renal venous prostaglandin

Table 4 Estimated prostaglandin (PG) secretion rates (ng/min) in kidneys from control and experimental rats

	PGE_2		$PGF_{2\alpha}$	
Group	*Left kidney*	*Right kidney*	*Left kidney*	*Right kidney*
Control	8.68	8.71	5.86	5.04
1	6.24	6.82	5.25	5.31
2	5.57*	8.06	4.28*	6.05
3	8.51	6.65	5.93	5.11
4	4.84	11.41	1.81	6.62
5	5.62	9.25	2.64	9.04
6	4.13*	5.51*	2.37*	2.75*
7	3.08	6.67	2.02	4.81

Values were obtained from the product of total mean RPF of each group and the respective prostaglandin concentration and are expressed in nanograms per minute. Groups 1 to 4 had left kidneys clipped. Groups 5 to 7 had both kidneys clipped.

* Signifies secretion rates where a statistically significant fall in RPF was observed.

E_2, as detected by radioimmunoassay, rose, but calculated secretion rate fell. We have also obtained similar results in the pump perfused canine kidney (Dighe, Hall, Smith and Ungar, unpublished observations). This is in contrast to what is generally believed and demonstrates the possible fallacy of equating concentration changes with secretion changes especially when concentration and flow change in an inverse manner as they do in the clamped kidney.

Our results also suggest that secretion rates of prostaglandin E_2 and $F_{2\alpha}$ fall in the chronically ischaemic rat kidney, with little change in the contralateral kidney. It must be borne in mind that our secretion figures were obtained from two groups of rats, namely those used for prostaglandin estimations and those for renal function tests, but there is no reason why these rats should not have been representative.

A reduced release of prostaglandin E-like material by the isolated kidney of one kidney Goldblatt rats, perfused at constant flow and challenged with pressor doses of noradrenaline, has been reported (Leary, Ledingham & Vane, 1974). Reduced synthetic capacity of prostaglandins was also found in the kidneys of renal and spontaneously hypertensive rats (Sirvios & Gagnon, 1974).

In contrast, using radioimmunoassay, Jaffe, Parker, Marshall & Needleman (1972) observed a rise in prostaglandin E in both the clamped and contralateral kidneys of chronic hypertensive rats. Arterial levels were also raised, suggesting a genuine increase in synthesis, but not necessarily from the kidney.

In earlier investigations (Somova, 1971; 1973) an increase in prostaglandin E-like material was seen in the ischaemic kidney of the rat during the acute phase of hypertension, with a decrease in the chronic stages.

The contralateral kidney has an endocrine antihypertensive role, distinct from excretion as demonstrated by Grollman *et al.* (1949) and Kolff (1958) in the dog. Similar findings have been reported in the rat kidney, when introduced into the circulation of the hypertensive rat (Tobian *et al.*, 1964). Muirhead, Germain, Brooks & Stephenson (1973) have attributed this property to the renal medulla and probably to the renal interstitial cells, which have been shown to synthesize prostaglandins. Rabbit renomedullary lipid is antihypertensive in rat (Muirhead, Leach, Daniels & Hinman, 1968) and rat renal medulla, especially that from hypertensive rats, is antihypertensive when implanted into hypertensive rats (Tobian & Azar, 1971). However, the antihypertensive principle may not be the renal prostaglandins but the neutral lipid (ANRL), which the renal interstitial cells also elaborate (Muirhead, Leach, Germain, Byers & Armstrong, 1974).

Certainly our results do not agree with the concept that the antihypertensive function of the kidney is due to an enhanced release of prostaglandin E_2 since secretion rate in the contralateral kidney does not change.

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References

ANDERSON, R.J., TAHER, M.S., CRONIN, R.E., McDONALD, K.M. & SCHRIER, R.W. (1975). Effect of β-adrenergic blockade and inhibitors of angiotensin II and prostaglandins on renal autoregulation. *Am. J. Physiol.*, **229**, 731–736.

ÅNGGÄRD, E., BOHMAN, S.O., GRIFFIN, J.E. III, LARSSON, C. & MAUNSBACH, A.B. (1972). Subcellular localization of the prostaglandin system in the rabbit renal papilla. *Acta physiol. scand.*, **84**, 231–246.

ÅNGGÄRD, E., LARSSON, C. & SAMUELSSON, B. (1971). The distribution of 15-hydroxy prostaglandin dehydrogenase and prostaglandin-Δ^{13}-reductase in tissues of the swine. *Acta physiol. scand.*, **81**, 396–404.

BECKMAN, M.L. & ZEHR, J.E. (1975). Partition of PGE between renal venous plasma and urine during renal ischaemia. *Prostaglandins*, **9**, 721–736.

BLATCHLEY, F.R., DONOVAN, B.T., HORTON, E.W. & POYSER, N.L. (1972). The release of prostaglandins and progestin into the utero-ovarian venous blood of guinea-pigs during the oestrous cycle and following oestrogen treatment. *J. Physiol. Lond.*, **223**, 69–88.

DIXON, W.J. & MASSAY, F.J. (1969). *Introduction to statistical analysis*. Third edition. p. 116. New York: McGraw-Hill Book Company.

GIRNDT, J. & OCHWADT, B. (1969). Durchblutung des Nierenmarkes, Gesamtnierendurchblutung und corticomedulläre Gradienten beim experimentallen renalen Hochdruck der Ratte. *Pflügers Archiv.*, **313**, 30–42.

GROLLMAN, A., MUIRHEAD, E.E. & VANATTA, J. (1949). Role of the kidney in the pathogenesis of hypertension as determined by a study of the effects of bilateral nephrectomy and other experimental procedures on the blood pressure of the dog. *Am. J. Physiol.*, **157**, 21–30.

HENSBY, C.N. & NAYLOR, B. (1974). The quantitative assay of prostaglandin E_2 and $F_{2\alpha}$ in biological extract using a Finnigan 3000D quadrupole mass spectrometer. *Br. J. Pharmac.*, **52**, 148P.

JAFFE, B.M., PARKER, C.W., MARSHALL, G.R. &

NEEDLEMAN, P. (1972). Renal concentration of PGE in acute and chronic renal ischaemia. *Biochem. biophys. Res. Commun.*, **49**, 799–805.

HERBACZYNSKA-CEDRO, K. & VANE, J.R. (1973). Contribution of intrarenal generation of prostaglandin to autoregulation of renal blood flow in dog. *Circulation Res.*, **33**, 428–436.

KOLFF, W.J. (1958). Reduction of experimental renal hypertension by kidney perfusion. *Circulation*, **17**, 702–707.

KRAMER, P. & OCHWADT, B. (1974). Separate renal concentrating ability in chronically hypertensive rats with unilateral Goldblatt clip. *Nephron*, **12**, 464–474.

LEARY, W.P., LEDINGHAM, J.G. & VANE, J.R. (1974). Imparied prostaglandin release from the kidneys of salt-loaded and hypertensive rats. *Prostaglandins*, **7**, 425–432.

LEE, J.B. (1973). Hypertension, natriuresis and the renomedullary prostaglandins. An overview. *Prostaglandins*, **3**, 551–579.

LONIGRO, A.J., TERRAGNO, N.A., MALIK, K.U. & McGIFF, J.C. (1973). Differential inhibition by prostaglandins of the renal actions of pressor stimuli. *Prostaglandins*, **3**, 595–606.

MALIK, K.U. & McGIFF, J.C. (1975). Modulation of prostaglandins of adrenergic transmission in the isolated perfused rabbit and rat kidney. *Circulation Res.*, **36**, 599–609.

McGIFF, J.C., CROWSHAW, K., TERRAGNO, R.A., LONIGRO, A.J., STRAND, J.C., WILLIAMSON, M.A., LEE, J.B. & NG, K.K.F. (1970). Prostaglandin-like substances appearing in the canine renal venous blood during renal ischaemia. *Circulation Res.*, **27**, 765–782.

McGIFF, J.C. & NASJLETTI, A. (1973). Renal prostaglandins and the regulation of blood pressure. In *Prostaglandins and cyclic AMP. Biological Actions and Clinical Applications*. ed. Kahl, R. & Lands, W. pp. 119–151. New York and London: Academic Press.

MERCER, P.F. (1971). Effects of bilateral vagotomy on renal function in the rat. *Can. J. Physiol. Pharmac.*, **49**, 420–426.

MUIRHEAD, E.E., GERMAIN, G.S., BROOKS, B. & STEPHEN, P. (1973). Renomedullary interstitial cells (RIC), prostaglandins (PG) and the antihypertensive function of the kidney. *Prostaglandins*, **3**, 581–594.

MUIRHEAD, E.E., LEACH, B.E., DANIELS, E.D. & HINMAN, J.W. (1968). Lapine renomedullary lipid in murine hypertension. *Arch. Path.*, **85**, 72–79.

MUIRHEAD, E.E., LEACH, B.E., GERMAIN, G.S., BYERS, L.W. & ARMSTRONG, F.B. (1974). Effect of indomethacin on antihypertensive lipids derived from renomedullary interstitial cells grown in tissue culture. *Fedn. Proc.*, **33**, 339.

OWEN, T., EHRHART, I., WEIDNER, J., HADDY, F. & SCOTT, J. (1974). Effects of indomethacin (I) on blood flow, reactive hyperaemia (RH) and autoregulation (AR) in the dog kidney. *Fedn. Proc.*, **33**, 348.

POYSER, N.L. (1972). Production of prostaglandins by the guinea-pig uterus. *J. Endocr.*, **54**, 147–159.

SIRVIOS, P. & GAGNON, D.J. (1974). Release of renomedullary prostaglandins in normal and hypertensive rats. *Experentia*, **30**, 1418–1419.

SOMOVA, L.I. (1971). A study of the vasodepressor lipid isolated from the kidneys of hypertensive animals. *Nephron*, **8**, 575–583.

SOMOVA, L.I. (1973). Inhibition of prostaglandin synthesis in the kidneys by aspirin-like drugs. *Adv. Biosci.*, **9**, 335–339.

THOMPSON, C.J., LOS, M. & HORTON, E.W. (1970). The separation, identification and estimation of prostaglandins in nanogram quantities by combined gas chromatography-mass spectrometry. *Life Sciences*, **9**, 983–988.

TOBIAN, L. & AZAR, S. (1971). Antihypertensive and other functions of the renal papilla. *Trans Am. Assoc. Physicians*, **84**, 281–288.

TOBIAN, L., SCHONNING, S. & SEEFELDT, C. (1964). The influence of arterial pressure on the antihypertensive action of a normal kidney, a biological servomechanism. *Ann. intern. Med.*, **60**, 378–383.

VENUTO, R.C., O'DORISIO, T., FERRIS, T.F. & STEIN, J.H. (1975). Prostaglandins and renal function II. The effect of prostaglandin inhibition on autoregulation of blood flow in the intact kidney of dog. *Prostaglandins*, **9**, 817–828.

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RENAL BLOOD FLOW AUTOREGULATION AND RENAL VENOUS PROSTAGLANDINS IN THE PUMP-PERFUSED CANINE KIDNEY (*In situ*)

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1 Renal autoregulation of blood flow was re-examined in the pump-perfused canine kidney and concentrations of prostaglandins E and F in the renal venous plasma were measured by radioimmunoassay.

2 At low perfusion pressures, below the range of autoregulation, prostaglandin E and F concentrations rose and calculated prostaglandin E secretion rate fell.

3 Meclofenamate (10 mg/kg, i.v.) reduced renal blood flow and prostaglandin E and F secretion rates, but did not abolish autoregulation.

4 Renal prostaglandins do not appear to mediate autoregulation in the kidney but may affect the level at which flow is controlled.

Introduction

The mechanism of renal blood flow autoregulation has escaped elucidation despite many attempts to implicate such factors as plasma skimming (Kinter & Pappenheimer, 1956), myogenic responses (Waugh, 1958) and tissue pressure (Hinshaw, Ballus, Day & Carlson, 1959). A capillaron model has also been proposed (Murao & Rodbard, 1976) but fails to predict why there is an upper limit to autoregulation. Herbaczynska-Cedro & Vane (1973), using pump-perfused kidneys, demonstrated the abolition of autoregulation and output of prostaglandin-like substances by indomethacin, implying prostaglandin E as a mediator of autoregulation. This was subsequently challenged. Owen, Ehrhart, Weidner, Haddy & Scott (1974) failed to abolish autoregulation in the naturally perfused kidney using indomethacin. Venuto, O'Dorisio, Ferris & Stein (1975) and Anderson, Taher, Cronin, McDonald & Schrier (1975) also failed to abolish autoregulation using meclofenamate as well as indomethacin.

This discrepancy has been assigned to a methodological difference, namely the large resting resistance to flow seen in the pump-perfused kidney (Venuto *et al.*, 1975; Anderson *et al.*, 1975). We re-examined the role of prostaglandins of the E and F series in the pump-perfused canine kidney using meclofenamate to inhibit synthesis, and radioimmunoassay to estimate renal venous concentrations of both prostaglandins.

Methods

Ten mongrel dogs of either sex, weighing between 8 and 15.5 kg were used. They were anaesthetized with pentobarbitone (30 mg/kg, i.v.) and surgical diathermy was used in subsequent operative procedures (there was no significant blood loss). Each animal was tracheotomized and systemic blood pressure was measured from the left femoral artery. Temperature was held at 37°C by a heating pad controlled by a thermistor rectal probe. A midline laparotomy was performed and a catheter placed in the left renal vein via a femoral vein, for renal venous blood sampling. The left ovarian or spermatic vein was ligated. Hypotonic saline (0.8% w/v NaCl solution) was infused intravenously at about 2 ml/min to ensure adequate urine flow. The animal was injected with heparin (1000 u/kg) and blood removed from the right carotid artery by a slowly revolving Watson Marlow MHRE pump. Part of this flow passed through a cannulating flow probe (Statham, 2.00 mm lumen) and was carried by silicone tubing through an incision in the abdominal wall to the catheterized left renal artery. Renal artery pressure (RAP) was measured at the tip of this catheter through a fine tube.

The remaining flow was shunted through a Starling resistance to a femoral vein. Using compressed air, with a controllable leak, the pressure in the Starling resistance box and so the mean perfusion pressure of the kidney could be controlled, while the pulse pressure remained about 25 mmHg. With this system

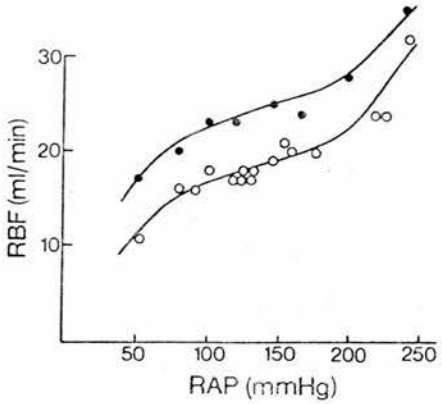

Figure 1 This figure shows the individual results of one experiment (expt. 7) before (●) and after (○) meclofenamate. RBF = renal blood flow; RAP = renal artery pressure. The lines through the points were obtained from the cubic expression of each set of points; $y = 24.9 + 0.040(x-141) + 5.26(x-141)^3$ for control and $y = 18.5 + 0.037(x-137) + 6.46(x-137)^3$ for the meclofenamate curve.

the kidney could be perfused at a range of pressures while the pump ran at a constant slow speed, thus preventing damage to the blood at high pump speeds.

All pressures were measured with Consolidated Electrodynamic L223 transducers and the electronically averaged pressures and renal blood flow signals were recorded on light-sensitive paper (Honeywell, Visicorder 2206). Pressure flow curves were obtained by varying RAP every 5 min, since autoregulation was complete within 3 minutes. After a control curve was obtained, meclofenamate (10 mg/kg, i.v.) dissolved in 10 ml of 0.9% saline was injected and after 15 to 20 min another pressure flow curve was recorded.

Samples of renal venous blood (10 ml) were removed at 50, 100, 150 and 200 mmHg during the determination of the control pressure flow curve and at 100 mmHg after meclofenamate in 4 experiments. The samples were collected in ice cold centrifuge tubes and spun at 4°C for 25 min at 15,000 *g*. The haematocrit of each sample was noted for estimation of renal plasma flow and so prostaglandin secretion rate. The plasma was stored in a freezer for less than 3 weeks before the assay. The plasma was acidified and the prostaglandins extracted and purified by silicic acid column chromatography before radioimmunoassay.

Prostaglandin antibody was raised in rabbits with prostaglandin E_2 conjugated to thyroglobulin by means of the carbodiimide reaction. The antibody exhibited a cross-reaction of 100% with prostaglandin E_1, 2% with prostaglandin A and B groups and 5% with 13, 14 dihydro-15-keto prostaglandin E_2 and was used at a final concentration of 1/2,000. The sensitivity was 30 pg/tube (60 pg/ml of sample), with an intra-assay precision of about 15%.

Prostaglandin F was measured by means of the antibody raised in rabbits to prostaglandin $F_{2\alpha}$ bovine serum albumin conjugate (Dighe, Emslie, Henderson, Rutherford & Simon, 1975). The antibody showed a cross-reaction of 100% with prostaglandin $F_{1\alpha}$, 2.6% with $F_{2\beta}$ and 2% with D_2. The other prostaglandin groups and metabolites cross-reacted less than 1%. The antibody was used at a final dilution of 1/35,000 and the sensitivity was 45 pg/tube (90 pg/ml of sample), with an intra-assay precision of about 15%. Recovery of both prostaglandins was about 60% and the reported levels were corrected for recovery.

The pressure flow curves were plotted for each individual experiment after fitting the data by computer to a cubic expression, which describes the general shape of the autoregulation curve:

$$y = p + q(x-m) + r(x-m)^3$$

where p is the flow during autoregulation (point of inflexion), m is the pressure at this point, q is the slope of the plateau part of the autoregulation curve, r is a constant, x is the pressure at any point and y is the flow at any point.

Figure 1 demonstrates the results of one experiment (expt. 7) to which the cubic expression has been fitted by the computer using the method of least squares.

A straight regression line was also fitted to the experimental points. In the absence of autoregulation, the plateau gradient (q) would approximate to the regression coefficient (β) such that q/β would tend to unity. Values of q/β less than one or even negative were taken as objective evidence for autoregulation.

Values are expressed as means ± s.e. and the results were analysed by Student's *t* test; probabilities less than 5% were accepted as being statistically significant.

Results

Mean systolic pressure (101 ± 5 mmHg) was well maintained throughout each experiment. Two experiments showed no autoregulation and a third lost autoregulation before completion of the control period. In the remaining 7 experiments autoregulation was obtained. Mean autoregulation flow was 54 ± 13 ml/min ($n=7$) and meclofenamate (10 mg/kg, i.v.) reduced it to 26 ± 6 ml/min ($n=6$, $P<0.05$). Autoregulation was lost in one experiment after meclofenamate. The mean autoregulation flow corrected for kidney weight was 1.6 ± 0.4 ml/min per g kidney, which is about 30% lower than that reported by Ono, Kokubun & Hashimoto (1974).

Midpoint autoregulation pressure (m) did not change after meclofenamate, 133 ± 5 mmHg before and 144 ± 10 mmHg after. The ratio q/β was always

less than unity before and after meclofenamate (except in one experiment where autoregulation was lost after meclofenamate), indicating preservation of autoregulation. See Table 1 for the haemodynamic data.

There was a tendency for renal venous prostaglandin E concentrations to rise and calculated secretion rates (the product of the concentrations and their respective renal plasma flows) to fall as RAP was reduced. Using absolute values no significance could be demonstrated due to the large variation in values between experiments (Table 2). By expressing the values at 50, 100 and 150 mmHg as ratios of those at 200 mmHg, a statistically significant rise in concentration of prostaglandin E was seen at the lowest pressure of 50 mmHg ($P<0.05$) and the calculated secretion rate fell ($P<0.01$).

Prostaglandin F concentrations expressed in a similar manner also rose as pressure fell, reaching statistical significance at the lowest pressure ($P<0.05$). However, prostaglandin F secretion did not change.

Meclofenamate (10 mg/kg) produced a significant

Table 1 Renal haemodynamics before and after meclofenamate

Experiment	*m*	*q*	*β*	*p*	*q/β*
1A	116	0.012	0.076	29.2	0.158
1B	132	0.057	0.095	13.4	0.600
2A	133	−0.027	0.036	25.4	−0.750
2B	184	0.025	0.033	10.7	0.757
3A	120	0.016	0.180	33.9	0.089
3B	155	0.147	0.175	25.9	0.840
4A	150	0.070	0.360	73.0	0.194
4B	113	0.111	0.229	41.5	0.484
5A	145	0.059	0.538	112.7	0.110
5B	144	0.048	0.130	46.3	0.369
6A	127	0.060	0.261	80.8	0.229
6B	(42)	(0.24)	0.17	(6.97)	(1.43)
7A	141	0.040	0.080	24.9	0.560
7B	137	0.037	0.083	18.5	0.446

A–control values; B–values after meclofenamate. m = autoregulation midpoint pressure (mmHg); q = slope of autoregulation point of curve; β = linear regression coefficient; p is the autoregulation flow (ml/min); q/β is an index of autoregulation. In the absence of autoregulation this value tends to unity.
In expt. 6 autoregulation was lost after meclofenamate and the pressure-flow curve becoming virtually rectilinear. The derived parameters except β have little meaning.

Table 2 Prostaglandin E (PGE) and prostaglandin F (PGF) renal venous plasma concentrations and calculated secretion rates at different renal artery pressures (RAP).

RAP (mmHg)	n	*PGE concentration*		*PGE secretion rate*	
		pg/ml	*ratios*	*ng/min*	*ratios*
50	6	542 ± 222	1.40 ± 0.17*	11.31 ± 4.83	0.65 ± 0.12†
100	5	311 ± 47	1.11 ± 0.25	10.77 ± 2.91	0.80 ± 0.21
150	5	398 ± 127	1.04 ± 0.17	19.47 ± 7.71	0.97 ± 0.25
200	6	360 ± 110	1	15.99 ± 4.63	1
		PGF concentration		*PGF secretion rate*	
		pg/ml	*ratios*	*ng/min*	*ratios*
50	4	800 ± 353	2.24 ± 0.49*	17.53 ± 7.24	1.04 ± 0.18
100	4	237 ± 67	1.07 ± 0.33	10.79 ± 3.84	0.92 ± 0.30
150	4	363 ± 105	1.33 ± 0.47	25.20 ± 9.07	1.82 ± 0.72
200	4	368 ± 173	1	16.37 ± 5.62	1

Absolute values are given and ratios relative to those values at 200 mmHg. *n* is the number of observations.
* $P<0.05$ † $P<0.01$

fall in the calculated secretion rate of prostaglandin E, at a perfusion pressure of 150 mmHg, from 15.65 ± 4.43 ng/min ($n=8$) to 3.61 ± 0.61 ng/min ($n=8$, $P<0.05$). Renal venous plasma concentration also fell, from 315 ± 58 pg/ml to 178 ± 26 pg/ml ($P<0.05$). Prostaglandin F showed a similar response, with a fall in the secretion rate from 18.93 ± 5.44 ng/min ($n=7$) to 2.73 ± 0.27 ng/min ($n=7$, $P<0.01$) and in concentration from 314 ± 57 pg/ml to 134 ± 23 pg/ml ($P<0.01$).

Discussion

Renal blood flow in the anaesthetized dog is dependent on prostaglandin synthesis, particularly of prostaglandin E_2 (Lonigro, Itskovitz, Crowshaw & McGiff, 1973). Infusion of the prostaglandin E and A series into the canine renal artery increases renal blood flow (Fülgraff, Brandenbusch & Heintze, 1974; Tannenbaum, Splawinskii, Oates & Nies, 1975). Arachidonic acid also elevates flow and prostaglandin synthesis inhibition prevents this (Tannenbaum *et al.*, 1975).

Renal prostaglandins also appear to be involved in intrarenal blood flow distribution in the dog (Itskovitz, Stemper, Pacholczyk & McGiff, 1973; Chang, Splawinskii, Oates & Nies, 1975) and in the rabbit (Larsson & Ånggärd, 1974).

Acute renal ischaemia increases prostaglandin E-like material present in the renal vein (McGiff, Crowshaw, Terragno, Lonigro, Strand, Williamson, Lee & Ng, 1970). Herbaczynska-Cedro & Vane (1973) confirmed this and showed that indomethacin prevented this rise in concentration and also prevented autoregulation. This latter study implied that prostaglandin E was released in response to a lowered RAP and that this mediated autoregulation.

Beckman & Zehr (1975), using radioimmunoassay, showed that renal venous prostaglandin E concentration rose when renal blood flow was reduced, but this was due to a dilution effect. A 50% reduction in flow resulted in a rise in concentration but less than double the control concentration, which must be interpreted as a fall in secretion rate.

In calculating secretion rate, it is assumed that the arterial prostaglandins presented to the kidney are removed in passage (Aiken & Vane, 1973) so that all the renal venous prostaglandins can be assumed to have been newly synthesized by the kidney. It is possible that the intrarenal metabolism of prostaglandins is flow-dependent, decreasing in efficiency as the flow increases.

In the naturally perfused kidney, prostaglandin synthetase inhibitors do not abolish renal blood flow autoregulation (Owen *et al.*, 1974; Venuto *et al.*, 1975; Anderson *et al.*, 1975). The latter two groups suggest that the large resting resistance seen in the pump-perfused kidney (Herbaczynska-Cedro & Vane, 1973) may lead to prostaglandin-dependent autoregulation. This cannot be the case since despite inhibition of prostaglandin synthesis, 6 out of 7 of our experiments still showed autoregulation. Prostaglandin E secretion was reduced by about 70%, in agreement with Venuto *et al.* (1975). It is possible that although secretion rate was greatly reduced, the smaller reduction in concentration (due to the concomitant fall in flow) was not enough to abolish autoregulation. It is possible that intrarenal blood prostaglandin concentration rather than secretion rate *per se* is important in determining intrarenal haemodynamics.

In the conscious dog, indomethacin (2 mg/kg) did not affect blood pressure, renal blood flow or intrarenal blood flow distribution (Zins, 1975) and meclofenamate and the competitive synthetase inhibitor Ro 20–5720 (1 mg/kg) did not alter blood pressure or renal blood flow (Kirschenbaum & Stein, 1976). However, in these studies, inhibition was not examined.

Renal blood flow and intrarenal haemodynamics may be dependent on prostaglandin synthesis in the anaesthetized dog due to the elevated prostaglandin E concentrations. In 7 conscious dogs we have obtained resting renal venous plasma concentrations of prostaglandin E of 191 ± 25 pg/ml ($n=10$), levels significantly lower than those reported in the anaesthetized dogs at a pressure of 100 mmHg, 361 ± 63 pg/ml ($n=10$, $P<0.05$). Levels may be higher in the anaesthetized animal for several reasons. The high resting resistance in the pump-perfused kidney will lower flow and so raise concentrations through a diluting mechanism. Angiotensin is released during barbiturate anaesthesia and this lowers renal blood flow (Burger, Hopkins, Tulloch & Hollenburg, 1976) and will also cause a direct release of prostaglandins (McGiff, Crowshaw, Terragno & Lonigro, 1970).

The mere introduction of the superfusion bioassay system as used by Herbaczynska-Cedro & Vane (1973) has been reported to elevate arterial and renal venous prostaglandin E, possibly due to kinin formation (Satch & Zimmerman, 1976). It is possible that foreign surfaces in general, such as those of the pump circuit tubing, may have a similar effect.

In conclusion, renal prostaglandins do not appear to mediate autoregulation of renal blood flow in the pump-perfused kidney, but may play a role in determining absolute blood flow, at least in the anaesthetized dog.

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References

References

AIKEN, J.W. & VANE, J.R. (1973). Intrarenal prostaglandin release attenuates the renal vasoconstrictor activity of angiotensin. *J. Pharmac. exp. Ther.*, **184**, 678–687.

ANDERSON, R.J., TAHER, M.S., CRONIN, R.E., McDONALD, K.M., & SCHRIER, R.W. (1975). Effect of β-adrenergic blockade and inhibitors of angiotensin II and prostaglandins on renal autoregulation. *Am. J. Physiol.*, **229**, 731–736.

BECKMAN, M.L. & ZEHR, J.E. (1975). Partition of PGE between renal venous plasma and urine during renal ischaemia. *Prostaglandins*, **9**, 721–736.

BURGER, B.M., HOPKINS, T., TULLOCH, A. & HOLLENBURG, N.K. (1976). The role of angiotensin in the canine renal vascular response to barbiturate anaesthesia. *Circulation Res.*, **38**, 196–202.

CHANG, L.C.T., SPLAWINSKII, J.A., OATES, J.A. & NIES, A.S. (1975). Enhanced renal prostaglandin production in the dog. II Effects on intrarenal haemodynamics. *Circulation Res.*, **36**, 204–207.

DIGHE, K.K., EMSLIE, H.A., HENDERSON, L.K., RUTHERFORD, F. & SIMON, L. (1975). The development of antisera to prostaglandins B_2 and $F_{2\alpha}$ and their analysis using solid-phase and double antibody radioimmunoassay methods. *Br. J. Pharmac.*, **55**, 503–514.

FÜLGRAFF, G., BRANDENBUSCH, G. & HEINTZE, K. (1974). Dose response relation of the renal effects of PGA_1, PGE_2 and $PGF_{2\alpha}$ in dogs. *Prostaglandins*, **8**, 21–30.

HERBACZYNSKA-CEDRO, K. & VANE, J.R. (1973). Contribution of intrarenal generation of prostaglandin to autoregulation of renal blood flow in the dog. *Circulation Res.*, **33**, 428–436.

HINSHAW, L.B., BALLUS, H.M., DAY, S.B. & CARLSON, C.H. (1959). Tissue pressure as a causal factor in the autoregulation of blood flow in the isolated perfused kidney. *Am. J. Physiol.*, **197**, 309–312.

ITSKOVITZ, H.D., STEMPER, J., PACHOLCZYK, D. & McGIFF, J.C. (1973). Determinants of intrarenal distribution of blood flow in the dog. *Clin. Sci. mol. Med.*, **45**, 321S–324S.

KINTER, W.B. & PAPPENHEIMER, J.R. (1956). The role of red blood cells in the regulation of renal blood flow and glomerular filtration rate. *Am. J. Physiol.*, **185**, 399–406.

KIRSCHENBAUM, M.A. & STEIN, J.H. (1976). The effect of inhibition of prostaglandin synthesis on urinary sodium excretion in the conscious dog. *J. clin. Invest.*, **57**, 517–521.

LARSSON, C. & ANGGÄRD, E. (1974). Increased juxtamedullary blood flow on stimulation of intrarenal prostaglandin biosynthesis. *Eur. J. Pharmac.*, **25**, 326–334.

LONIGRO, A.J., ITSKOVITZ, H.D., CROWSHAW, K. & McGIFF, J.C. (1973). Dependency of renal blood flow on prostaglandin synthesis in the dog. *Circulation Res.*, **32**, 712–717.

McGIFF, J.C., CROWSHAW, K., TERRAGNO, A.A., LONIGRO, A.J. STRAND, J.C., WILLIAMSON, M.A., LEE, J.B. & NG, K.K.F. (1970). Prostaglandin-like substances appearing in canine renal venous blood during renal ischaemia. Their partial characterization by pharmacologic and chromatographic procedures. *Circulation Res.*, **27**, 765–781.

McGIFF, J.C., CROWSHAW, K., TERRAGNO, N.A. & LONIGRO, A.J. (1970). Release of prostaglandin-like substances into renal venous blood in response to angiotensin II. *Circulation Res.*, **27**, suppl. 1, 121–130.

MURAO, H. & RODBARD, S. (1976). Mathematical formulation of post-occlusion hyperaemia and autoregulation of blood flow in the capillaron model. *Cardiovasc. Res.*, **10**, 13–19.

ONO, H., KOKUBUN, H. & HASHIMOTO, K. (1974). Abolition by calcium antagonists of the autoregulation of renal blood flow. *Arch Pharmacol.*, **285**, 201–207.

OWEN, T., EHRHART, I., WEIDNER, J., HADDY, F. & SCOTT, J. (1974). Effect of indomethacin on blood flow, reactive hyperaemia and autoregulation in the dog kidney. *Fedn Proc.*, **33**, 348.

SATCH, S. & ZIMMERMAN, B.G. (1976). Renal effect of meclofenamate in presence and absence of superfusion bioassay. *Am. J. Physiol.*, **230**, 711–714.

TANNENBAUM, J., SPLAWINSKII, J.A., OATES, J.A. & NIES, A.S. (1975). Enhanced prostaglandin production in the dog. I. Effects on renal function. *Circulation Res.*, **36**, 197–203.

VENUTO, R.C., O'DORISIO, T., FERRIS, T.F. & STEIN, J.H. (1975). Prostaglandin and renal function II. The effect of prostaglandin inhibition on autoregulation of blood flow in the intact kidney of dog. *Prostaglandins*, **9**, 817–828.

WAUGH, W.H. (1958). Myogenic nature of autoregulation of renal blood flow in the absence of blood corpuscles. *Circulation Res.*, **6**, 363–371.

ZINS, G.R. (1975). Renal prostaglandins. *Am. J. Med.*, **58**, 14–24.

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References

AIKEN, J.W. & VANE, J.R. (1973). Intrarenal prostaglandin release attenuates the renal vasoconstrictor activity of angiotensin. *J. Pharmac. exp. Ther.*, **184**, 678–687.

ANDERSON, R.J., TAHER, M.S., CRONIN, R.E., McDONALD, K.M., & SCHRIER, R.W. (1975). Effect of β-adrenergic blockade and inhibitors of angiotensin II and prostaglandins on renal autoregulation. *Am. J. Physiol.*, **229**, 731–736.

BECKMAN, M.L. & ZEHR, J.E. (1975). Partition of PGE between renal venous plasma and urine during renal ischaemia. *Prostaglandins*, **9**, 721–736.

BURGER, B.M., HOPKINS, T., TULLOCH, A. & HOLLENBURG, N.K. (1976). The role of angiotensin in the canine renal vascular response to barbiturate anaesthesia. *Circulation Res.*, **38**, 196–202.

CHANG, L.C.T., SPLAWINSKII, J.A., OATES, J.A. & NIES, A.S. (1975). Enhanced renal prostaglandin production in the dog. II Effects on intrarenal haemodynamics. *Circulation Res.*, **36**, 204–207.

DIGHE, K.K., EMSLIE, H.A., HENDERSON, L.K., RUTHERFORD, F. & SIMON, L. (1975). The development of antisera to prostaglandins B_2 and $F_{2\alpha}$ and their analysis using solid-phase and double antibody radioimmunoassay methods. *Br. J. Pharmac.*, **55**, 503–514.

FÜLGRAFF, G., BRANDENBUSCH, G. & HEINTZE, K. (1974). Dose response relation of the renal effects of PGA_1, PGE_2 and $PGF_{2\alpha}$ in dogs. *Prostaglandins*, **8**, 21–30.

HERBACZYNSKA-CEDRO, K. & VANE, J.R. (1973). Contribution of intrarenal generation of prostaglandin to autoregulation of renal blood flow in the dog. *Circulation Res.*, **33**, 428–436.

HINSHAW, L.B., BALLUS, H.M., DAY, S.B. & CARLSON, C.H. (1959). Tissue pressure as a causal factor in the autoregulation of blood flow in the isolated perfused kidney. *Am. J. Physiol.*, **197**, 309–312.

ITSKOVITZ, H.D., STEMPER, J., PACHOLCZYK, D. & McGIFF, J.C. (1973). Determinants of intrarenal distribution of blood flow in the dog. *Clin. Sci. mol. Med.*, **45**, 321S–324S.

KINTER, W.B. & PAPPENHEIMER, J.R. (1956). The role of red blood cells in the regulation of renal blood flow and glomerular filtration rate. *Am. J. Physiol.*, **185**, 399–406.

KIRSCHENBAUM, M.A. & STEIN, J.H. (1976). The effect of inhibition of prostaglandin synthesis on urinary sodium excretion in the conscious dog. *J. clin. Invest.*, **57**, 517–521.

LARSSON, C. & ANGGÄRD, E. (1974). Increased juxtamedullary blood flow on stimulation of intrarenal prostaglandin biosynthesis. *Eur. J. Pharmac.*, **25**, 326–334.

LONIGRO, A.J., ITSKOVITZ, H.D., CROWSHAW, K. & McGIFF, J.C. (1973). Dependency of renal blood flow on prostaglandin synthesis in the dog. *Circulation Res.*, **32**, 712–717.

McGIFF, J.C., CROWSHAW, K., TERRAGNO, A.A., LONIGRO, A.J. STRAND, J.C., WILLIAMSON, M.A., LEE, J.B. & NG, K.K.F. (1970). Prostaglandin-like substances appearing in canine renal venous blood during renal ischaemia. Their partial characterization by pharmacologic and chromatographic procedures. *Circulation Res.*, **27**, 765–781.

McGIFF, J.C., CROWSHAW, K., TERRAGNO, N.A. & LONIGRO, A.J. (1970). Release of prostaglandin-like substances into renal venous blood in response to angiotensin II. *Circulation Res.*, **27**, suppl. 1, 121–130.

MURAO, H. & RODBARD, S. (1976). Mathematical formulation of post-occlusion hyperaemia and autoregulation of blood flow in the capillaron model. *Cardiovasc. Res.*, **10**, 13–19.

ONO, H., KOKUBUN, H. & HASHIMOTO, K. (1974). Abolition by calcium antagonists of the autoregulation of renal blood flow. *Arch Pharmacol.*, 285, 201–207.

OWEN, T., EHRHART, I., WEIDNER, J., HADDY, F. & SCOTT, J. (1974). Effect of indomethacin on blood flow, reactive hyperaemia and autoregulation in the dog kidney. *Fedn Proc.*, **33**, 348.

SATCH, S. & ZIMMERMAN, B.G. (1976). Renal effect of meclofenamate in presence and absence of superfusion bioassay. *Am. J. Physiol.*, 230, 711–714.

TANNENBAUM, J., SPLAWINSKII, J.A., OATES, J.A. & NIES, A.S. (1975). Enhanced prostaglandin production in the dog. I. Effects on renal function. *Circulation Res.*, **36**, 197–203.

VENUTO, R.C., O'DORISIO, T., FERRIS, T.F. & STEIN, J.H. (1975). Prostaglandin and renal function II. The effect of prostaglandin inhibition on autoregulation of blood flow in the intact kidney of dog. *Prostaglandins*, **9**, 817–828.

WAUGH, W.H. (1958). Myogenic nature of autoregulation of renal blood flow in the absence of blood corpuscles. *Circulation Res.*, **6**, 363–371.

ZINS, G.R. (1975). Renal prostaglandins. *Am. J. Med.*, **58**, 14–24.

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The potentiation of exogenous noradrenaline by prostaglandins $F_{2\alpha}$ C_2 and D_2 on the canine saphenous vein

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Kadowitz, Sweet & Brody (1971) have shown that prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) potentiates the response of venous smooth muscle to exogenous noradrenaline (NA). PGD_2 acts similarly to $PGF_{2\alpha}$ on several preparations (Horton & Jones, 1974) whilst PGC_2 has similar actions to PGE_2 (Jones, Kane & Ungar, 1974). Experiments are reported here which compare the potencies of $PGF_{2\alpha}$, PGD_2 and PGC_2 on a single preparation.

The method used was a modification of that described by Webb-Peploe & Shepherd (1968) and was used to determine the venous response to exogenous NA before and during infusion of the various PGs. Both lateral saphenous veins were cannulated at the ankle and perfused separately, one serving as a control for the other. NA was injected proximal to the pump and once a dose dependent relationship was obtained, the PG infusion was commenced.

$PGF_{2\alpha}$ (1 μg/min) caused a significant potentiation of the response and the calculated potency ratio (R) from the pooled results of three preparations was 6. PGD_2 (1 μg/min) was not as potent (R = 3, from six preparations) and PGC_2 (1 μg/min) was the least potent (R = 2, from three preparations). Using linear regression analysis, the results during PG infusion (1 μg/min) were significantly different from control ($P < 0.05$). In the two preparations, $PGF_{2\alpha}$ (0.1 μg/min) was found to potentiate the response (R = 2). However, at 5 μg/min there were considerable baseline changes and no satisfactory tests were performed. At 5 μg/min PGC_2 caused similar baseline changes. Up to 10 μg/min, PGD_2 was apparently no more potent than at 1 μg/min and no baseline shift occurred.

The results with $PGF_{2\alpha}$ are largely in agreement with those of Kadowitz *et al.* (1971) and a similar potency ratio for $PGF_{2\alpha}$ (1 μg/min) has been obtained. We have found PGD_2 to be considerably less potent than $PGF_{2\alpha}$, whereas it is up to 60 times more potent than $PGF_{2\alpha}$ as a direct vasoconstrictor in the sheep (Jones, 1975). PGC_2 which from our results is only weakly active in potentiating the effects of NA, is in contrast a potent dilator of both resistance and capacitance vessels in the dog (Jones, Kane & Ungar, 1974).

References

HORTON, E.W. & JONES, R.L. (1974). Biological activity of prostaglandin D_2 on smooth muscle. *Br. J. Pharmac.*, **52**, 110–111P.

JONES, R.L. (1975). Actions of prostaglandins on the arterial system of the sheep: some structure-activity relationships. *Br. J. Pharmac.*, **53**, 464P.

JONES, R.L., KANE, A.K. & UNGAR, A. (1974). Cardiovascular actions of PGC_2 in the cat and dog. *Br. J. Pharmac.*, **51**, 157–160.

KADOWITZ, P.J., SWEET, C.S. & BRODY, M.J. (1971). Potentiation of adrenergic venomotor responses by Angiotensin, $PGF_{2\alpha}$ and cocaine. *J. Pharmac. exp. Ther.*, **176**, 167.

WEBB-PEPLOE, M.M. & SHEPHERD, J.T. (1968). Response of large hindlimb veins of the dog to sympathetic nerve stimulation. *Am. J. Physiol.*, **215**, 299–307.